

DEMOGRAPHY, TOPGRAPHIC ORIENTATION, AND MIGRATORY
PATTERNS OF TWO AMBYSTOMATID COMMUNITIES ON THE
SOUTHERN CUMBERLAND PLATEAU IN FRANKLIN COUNTY,
TENNESSEE

by

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Abstract

DEMOGRAPHY, TOPOGRAPHIC ORIENTATION, AND MIGRATORY PATTERNS OF TWO AMBYSTOMATID COMMUNITIES ON THE SOUTHERN CUMBERLAND PLATEAU IN FRANKLIN COUNTY, TENNESSEE

Habitat degradation and loss has been identified as one of the major causes of amphibian declines worldwide. Alterations to the terrestrial landscape can alter or eliminate migrations of amphibians and disrupt amphibian assemblages. Degradation of the upland habitat surrounding wetlands can have severe consequences for emigrating juvenile amphibians, decreasing gene flow within metapopulations. Analyses of topographic orientation of migratory amphibians can indicate habitat used and the occurrence migratory corridors on the landscape. This study occurred simultaneously at two wetlands located on the Southern Cumberland Plateau. The primary goals were to (1) determine the amphibian and reptile assemblages, (2) determine if migrations of ambystomatid salamanders were uniformly oriented, and (3) determine, through the use of telemetry, if current habitat management is impacting salamander migration patterns.

Two wetlands, 26007 and 26031, of differing habitats on Bear Hollow Mountain Wildlife Management Area were completely encircled with polypropylene fencing material. Pitfalls and box-style funnel traps were placed along both sides of the drift fence. Demographic and topographic orientation data were gathered between 07 December 2007 and 04 May 2010, encompassing a minimum of two breeding seasons for ambystomatid salamanders. Telemetry data were collected during the spring of 2011.

Four species of ambystomatid salamanders were captured at each site: *Ambystoma opacum*, *Ambystoma maculatum*, *Ambystoma talpoideum*, and *Ambystoma tigrinum*.

Ambystomatid salamanders dominated the capture total at each site. Size and ratio of males to females varied between sites. Female *A. opacum* were larger than males at 26007, but males outnumbered females at the site. Male *A. opacum* were larger and occurred with less frequency than females at 26031. Female *A. maculatum* were larger and outnumbered males at both 26007 and 26031. Male *A. talpoideum* were larger than females at both sites and out outnumbered females at 26007, but not 26031. Recruitment of juvenile ambystomatid salamanders was stochastic between species, sites, and years.

The uniformity of migratory patterns for both male and female *A. opacum* varied between years at site 26007. Female and male *A. maculatum* immigrations were not uniform at site 26007, but emigrations were uniformly distributed across the site. Uniform migrations varied across years for male and female *A. talpoideum*, but emigrations were uniformly distributed across site 26007.

The immigration of male, female, and emigration of adult *A. opacum* were not uniform at 26031. Migratory patterns of male and female *A. maculatum* were not uniform at 26031, but uniform emigration was observed during Year 3. *A. talpoideum* male and female migrations were uniformly distributed at site 26031.

Straight-line migratory distances of *A. maculatum* averaged 388.18 m (range 80 – 840 m) at site 26007 and 310.67 m (range 94 – 480 m) at site 26031. The terrestrial buffer zone at each site necessary to protect 95% of the breeding population is 557.89 m (97.78 ha) at site 26007 and 439.27 m (60.62 ha) at site 26031.

Surveys indicate that large populations of ambystomatid salamanders exist in the terrestrial habitat surrounding each site. The size of these populations varied between sites and the habitat modifications surrounding site 26031 potentially impacting juvenile recruitment resulting in the differences in population sizes detected; however, it is not known if habitat management is the sole reason for differences in population size. Migratory distances observed during telemetry are the longest recorded for *A. maculatum* and indicate this species can migrate across open habitat during one night. Although *A. maculatum* has the ability to migrate across open habitat given certain environmental conditions exist, the removal of vast areas of surrounding terrestrial habitat may prove detrimental to juvenile recruitment and the overall population stability.

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Introduction

Declines in amphibian populations have been documented for almost forty years (Stuart *et al.* 2004). Introduction of invasive species (Gamradt and Kats 1996), ultraviolet radiation (Blaustein *et al.* 1994; Long *et al.* 1995; Kiesecker *et al.* 2001; Lesser *et al.* 2001), global climate change (Wyman 1990), disease (Briggs *et al.* 2005), and pollution (Hopkins *et al.* 1997) are just a few of the many causes of amphibian declines worldwide. However, habitat fragmentation, degradation, and loss is considered the greatest threat to species extinction (Fahrig 2001; Young *et al.* 2004; Brodman *et al.* 2006; Dodd 2010). Human population growth is accompanied by the expansion of land use and development, resulting in fragmentation of the landscape. Wilcox and Murphy (1985) noted two components of habitat fragmentation: habitat loss and insularity of fragments. Both components contribute to loss in biodiversity. The potential effects of fragmentation can be the reduction, subdivision or extinction of demographic units, loss of the source of immigrants, and the impediment of immigration through the conversion of habitat between suitable patches (Wilcox and Murphy 1985; Torrence 2007). Furthermore, these effects can combine with stochastic natural events to eliminate fragmented populations from the landscape (Shafer 1981). Ficetola and De Bernardi (2004) found that species richness increased as the effect of fragmentation on wetlands within the landscape decreased. The complexities of species life cycles compound the effects of habitat fragmentation, degradation, and loss. For instance, many amphibians have complex life cycles requiring terrestrial habitat during non-breeding portions of the year and aquatic habitat for breeding and subsequent development of larvae. Diphasic

life cycles such as these require conservation of distinctly different habitats within the landscape.

Stuart *et al.* (2004) identified the family Ambystomatidae as one of the most rapidly declining taxonomic groups of amphibians. More than 44% of ambystomatid species are experiencing some form of decline. Losses of both terrestrial and aquatic habitats through fragmentation or degradation have had negative impacts on pond-breeding amphibians (Petranka 1998). Between 1960 and 1990, the amount of forested wetlands in the United States was reduced by three million ha, and during the 1980's the loss of this habitat was almost twice the reduction in forestland (Rader *et al.* 2001). Mitsch and Gosselink (2007) identified the need for protection of small wetlands, as these were most vulnerable to being lost. Windmiller *et al.* (2008) documented the extirpation of a local population of Wood Frogs, *Rana sylvatica*, following the loss of forested habitat, illustrating the impact of reduction in non-breeding habitat.

To conserve populations of ambystomatid salamanders, preservation of both aquatic and terrestrial habitat must occur (Porej *et al.* 2004). Biological delineation of terrestrial "core zones" facilitates the preservation of habitat surrounding breeding sites of pond breeding salamanders (Semlitsch 1998; Semlitsch and Jensen 2001; Semlitsch and Bodie 2003). Widths of core zones are determined through the evaluation of emigration distances of pond breeding amphibians from aquatic habitat. Semlitsch (1998) and Semlitsch and Bodie (2003) evaluated terrestrial habitat surrounding wetlands and suggest core terrestrial zones should extend circumferentially 218 m from the wetland edge for pond breeding salamanders. Porej *et al.* (2004) found a positive association between the amount of forest cover and the presence of four species of

ambystomatid salamanders. Diversity of pond-breeding amphibians is lower in wetlands surrounded by relatively little forest (Windmiller *et al.* 2008).

To adequately maintain populations of pond breeding amphibians, habitat management should conserve a diversity of terrestrial habitats and aquatic habitats that vary in hydroperiod (Semlitsch 2003). Although conservation of diverse habitats is important, minimizing or eliminating fragmentation of habitat ensures corridors exist allowing for dispersal and recolonization, and, therefore, reduces the likelihood of extirpation associated with human disturbances (Wilcox and Murphy 1985; Semlitsch 2003; Shaffer 1981). Without preservation of ample habitat, forestry practices can have negative impacts on amphibian populations inhabiting wetland ecosystems. Knapp (1999) indicated four impacts of timber harvesting on salamander habitat including (1) increases in air and soil temperatures, (2) desiccation of detritus, (3) increases or decreases in invertebrate biomass, and (4) a decline in the nutritional quality of invertebrates.

The effects of habitat alteration, degradation and loss on ambystomatid populations are wide ranging and include changes in population size, reproductive rate, age distribution, juvenile survival, and species richness (Knapp 1999). Clear-cutting of terrestrial habitat causes reductions in the quantity and quality of adult Spotted Salamanders, *Ambystoma maculatum*, indicating that habitat management affects the population biology of this species (Silver *et al.* 1999). Capture rates of Marbled Salamanders, *Ambystoma opacum*, are positively associated with canopy cover (Jenkins *et al.* 2006). Rothermel and Luhring (2005) suggest 60% of juvenile mortality in Mole

Salamanders, *Ambystoma talpoideum*, emigrating from wetlands is the result of habitat alteration resulting from clear-cutting.

Currently, government and non-government agencies have developed management plans for a variety of vertebrates that seek to restore populations of particular species without identifying the potential impacts of these actions on other faunal groups within restoration areas. For example, the Northern Bobwhite Conservation Initiative (NBCI) (Dimmick *et al.* 2002) seeks to restore populations of Northern Bobwhite to levels similar to those recorded in 1980. This initiative seeks to impact more than 32 million hectares of farm, forest, and range land habitat across the southeast (Dimmick *et al.* 2002). Although this plan seeks to create habitat preferred by Northern Bobwhites (e.g. early successional and old field habitats), no consideration is given to how an effort such as this will impact populations of amphibians. Giocomo *et al.* (2009) called for an integration of grassland and shrubland bird conservation with the NBCI to benefit quail and grassland and shrubland bird species. Although integration of multiple species habitat preferences would allow for conservation of multiple species, conversion of the necessary habitat to obtain goals of the management plan may have negative impacts on and lessen conservation efforts for amphibians.

Habitat management plans should incorporate species demographic and seasonal activity patterns to ascertain the potential negative impacts on species diversity (MWPARC 2009). To minimize impact on species diversity, management plans should incorporate connectivity between habitats and maintenance of natural hydroperiods (Buhlmann and Mitchell 2000). Slight alterations to management plans can lessen negative impacts to pond-breeding amphibians. For example, Dechant (2007) found

controlled burns in managed forests during autumn prior to leaf drop would still provide the needed detritus for fall migrations of pond-breeding salamanders. Temporary road closures were proposed by Timm *et al.* (2007a) to minimize mortality during nights of mass migrations. Baldwin *et al.* (2006) recommend accentuating as key habitats seasonal pools that contain water for 16-17 weeks post-breeding. Hydroperiods of this duration provide source populations capable of enduring harsh climatic conditions (e.g. drought).

Despite the numerous ambystomatid studies that have occurred nationally, data pertaining to the demographics and phenologies of ambystomatid populations within Tennessee are scarce. To date, two studies (Bailey and Bailey 2000; Wyckoff 2006) have been conducted on ambystomatid salamanders within the Eastern Highland Rim in which demographic and migratory patterns were assessed, but this study was concentrated in a single ecological region. Due to the paucity of data, it is unknown how the conversion of thousands of acres of forest habitat to grassland habitat within Tennessee would affect ambystomatid salamander populations.

The Tennessee Wildlife Resources Agency (TWRA) owns and manages 605,013 ha (Lavicot *et al.* 2010) within the nine physiographic provinces of the state (Smalley *et al.* 1996). These lands include wildlife management areas (WMA), refuges, and wetlands. Management decisions for these lands are habitat and species focused, and the decisions are based on scientific principles and public input (Tennessee Wildlife Resources Agency 2006). Impacts from habitat management may impact many more species than anticipated. In 2004, TWRA developed a Comprehensive Wildlife Conservation Strategy (CWCS), increasing its ability to manage rare and threatened species (Tennessee Wildlife Resources Agency 2005). As a part of the CWCS, a Geographic Information System

(GIS) database and model was developed to aid management decisions, land acquisition, environmental assessments, and policymaking. Although management tools created during the development of the CWCS have aided the agency's ability to manage nongame wildlife, continuing biological inventories and conducting research can contribute information allowing its enhancement and ensuring positive impacts are had on wildlife and fisheries management.

This study occurred simultaneously at two wetlands located on the Southern Cumberland Plateau. The primary goals were to (1) determine the amphibian and reptile assemblages, (2) determine if migrations of ambystomatid salamanders were uniformly oriented, and (3) determine, through the use of telemetry, if current habitat management is impacting salamander migration patterns. This research should aid land managers in determining how management may impact current amphibian populations (e.g. migration patterns and timing, habitat preferences, breeding times etc.) on the Southern Cumberland Plateau.

Study Site

The Southern Cumberland Plateau in Tennessee covers portions of Franklin and Marion counties in southern middle Tennessee: (Smalley 1979), which is characterized as having weakly dissected surface and strongly dissected margins and sides (Smalley 1982). Landtypes across this portion of the Southern Cumberland Plateau can be classified into three types, those occurring on the (1) top of the Plateau, (2) sides of the Plateau, and (3) those associated with the drainages (Smalley 1979). The tops of the Plateau contain broad undulating uplands, broad ridges with both north and south aspects, and plateau edges. Landtypes associated with the sides of the Plateau include the sandstone escarpment, talus slopes, benches with north and south aspects, and the lower slopes and benches with north and south aspects. Drainage landtypes include terraces, slopes and stream bottoms with both good and poor drainages.

Consistent with a temperate climate, summers may be long and hot, and winters are typically short and mild. This portion of the Southern Cumberland Plateau averages 200 or more frost-free days (Smalley 1979). The annual temperature averages 14.2 °C (Fox *et al.* 1958). Most rainfall occurs between December and March, and Fall is the driest period of the year (Fox *et al.* 1958). Annual precipitation averages 144.8 centimeters (Fox *et al.* 1958).

Bear Hollow Mountain Wildlife Management Area is located on the Southern Cumberland Plateau in Franklin County, Tennessee (Fig. 1). The WMA is made up of two compartments encompassing 6,879 ha of land. The location of the WMA is in the more highly dissected portion of the Southern Cumberland Plateau, characterized by deep

gorges, vertical escarpment, and undulating surfaces. Because of the dissection, differences in elevation may be as much as 800 feet within three-quarters of a mile (Fox *et al.* 1958). The management area is dominated by a mixed oak and oak-hickory forest on the Plateau top, with mixed mesophytic communities being restricted to coves and gorges (Smalley 1982), similar to other portions of the Southern Cumberland Plateau outside of Tennessee (Wang *et al.* 2010). Hartsells-Muskingum-Cotaco and Rockland, limestone Rockland, and sandstone-Stany soil associations dominate the WMA.

Located within, and sharing boundaries with Bear Hollow Mountain WMA, the Walls of Jericho State Natural Area (SNA) contains 303.5 hectares of highly dissected portions of the Southern Cumberland Plateau. This SNA boasts impressive geologic formations due to the natural processes caused by Turkey Creek that drains through the area. Numerous rare plant species occur across the SNA, and this is one of only three known locations of the state endangered Limerock Arrowwood (*Viburnum bracteatum*) (Tennessee Department of Environment and Conservation 2013). The Walls of Jericho SNA aids in the protection of Turkey Creek and the upper portion of the Upper Paint Rock watershed.

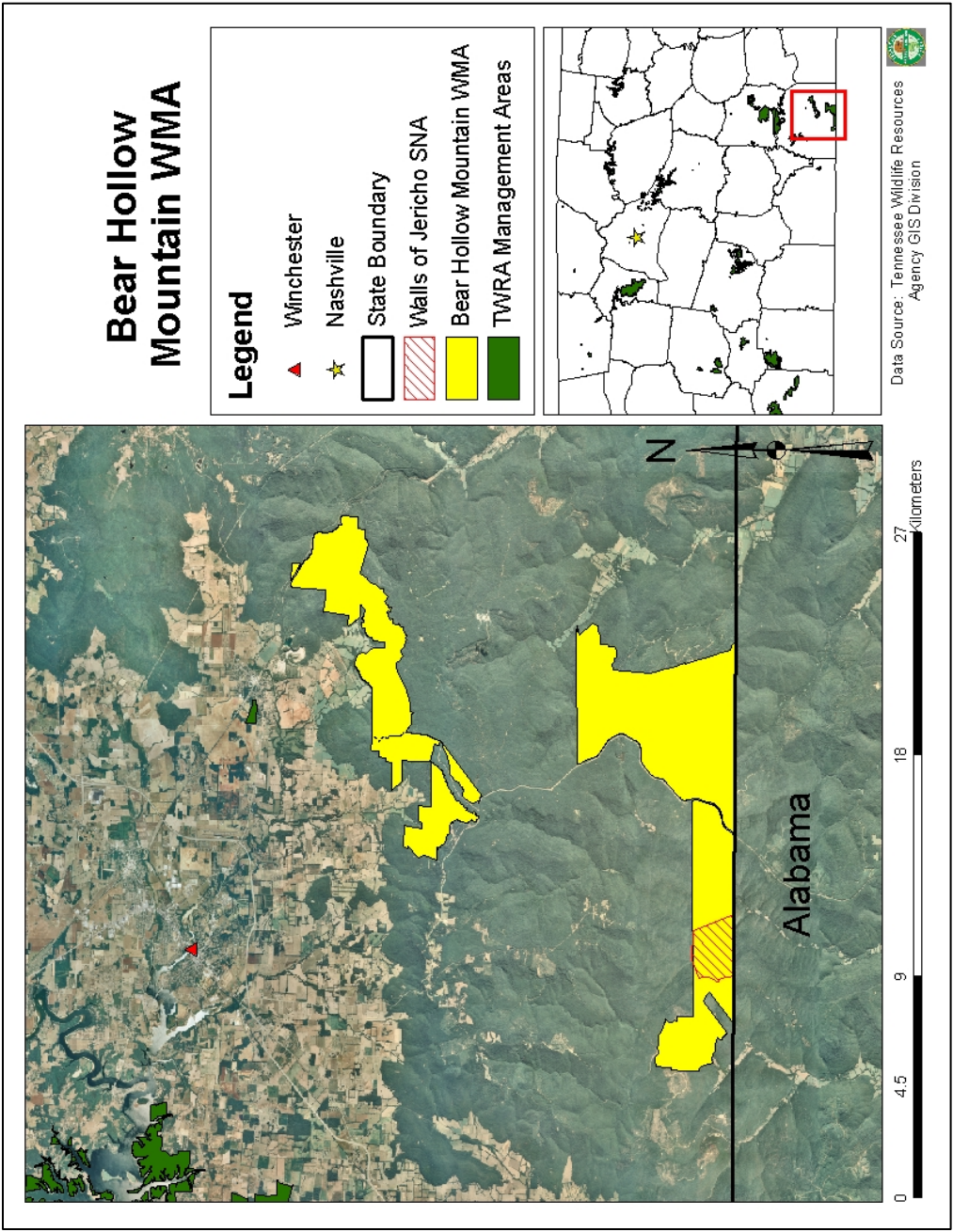


Figure 1. Location of Bear Hollow Mountain Wildlife Management Area on the Southern Cumberland Plateau in Franklin County, Tennessee.

Site Description

This study was conducted at two separate wetlands on the WMA, wetlands 26007 and 26031 (Fig. 2). Wetland 26031 was located approximately 2.4 km north of wetland 26007. Each wetland is filled from surface runoff from the surrounding habitat during the winter. The National Wetland Inventory (NWI), obtained from the TWRA Geographic Information Systems Division, identifies only eight wetlands on Bear Hollow Mountain WMA (Fig. 3); however, TWRA personnel have identified additional wetlands while conducting biological inventories on the WMA. Nonetheless, these additional wetlands cannot act as replacements to the NWI identified wetlands because of their scarcity, location within the landscape, brief hydroperiod, and physical characteristics. Although there is no scientifically accepted definition for isolated wetland (Leibowitz 2003), these sites are considered geographically isolated wetland systems, each being completely surrounded by upland habitat and having no surface water inlets or outlets (Comer *et al.* 2005), or direct connectivity to rivers and streams. Site 26031 contains an outlet along the western edge of the wetland, and water drains through the surrounding landscape when the wetland exceeds maximum capacity. Because of a lack of connectivity to any river or stream, the separation of more than 2,300 m, and the dissected Stubblefield Hollow, the amphibian populations inhabiting these wetlands were considered to be geographically isolated from each other (Fig. 2).

The maximum water depth recorded at 26007 was 0.95 m and at 26031, 1.08 m. The average depth of both sites was 0.6 m. Each site is surrounded by Hartsells fine sandy loam associated with the Muskingham and Cotaco soils of the upland portions of the Cumberland Plateau (Fox *et al.* 1958). Hartsells soils are strongly acidic and contain

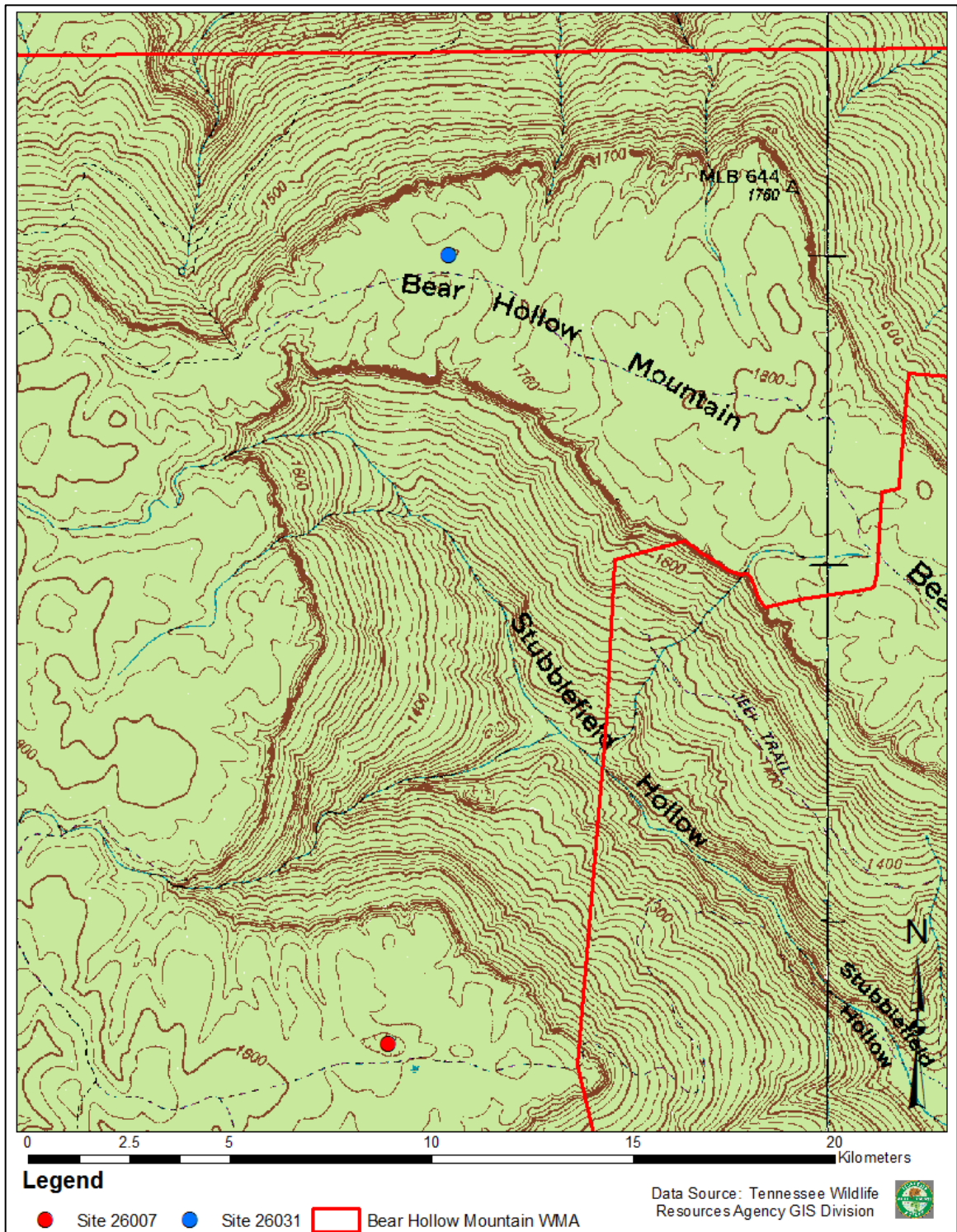


Figure 2: Location of survey sites on Bear Hollow Mountain WMA.

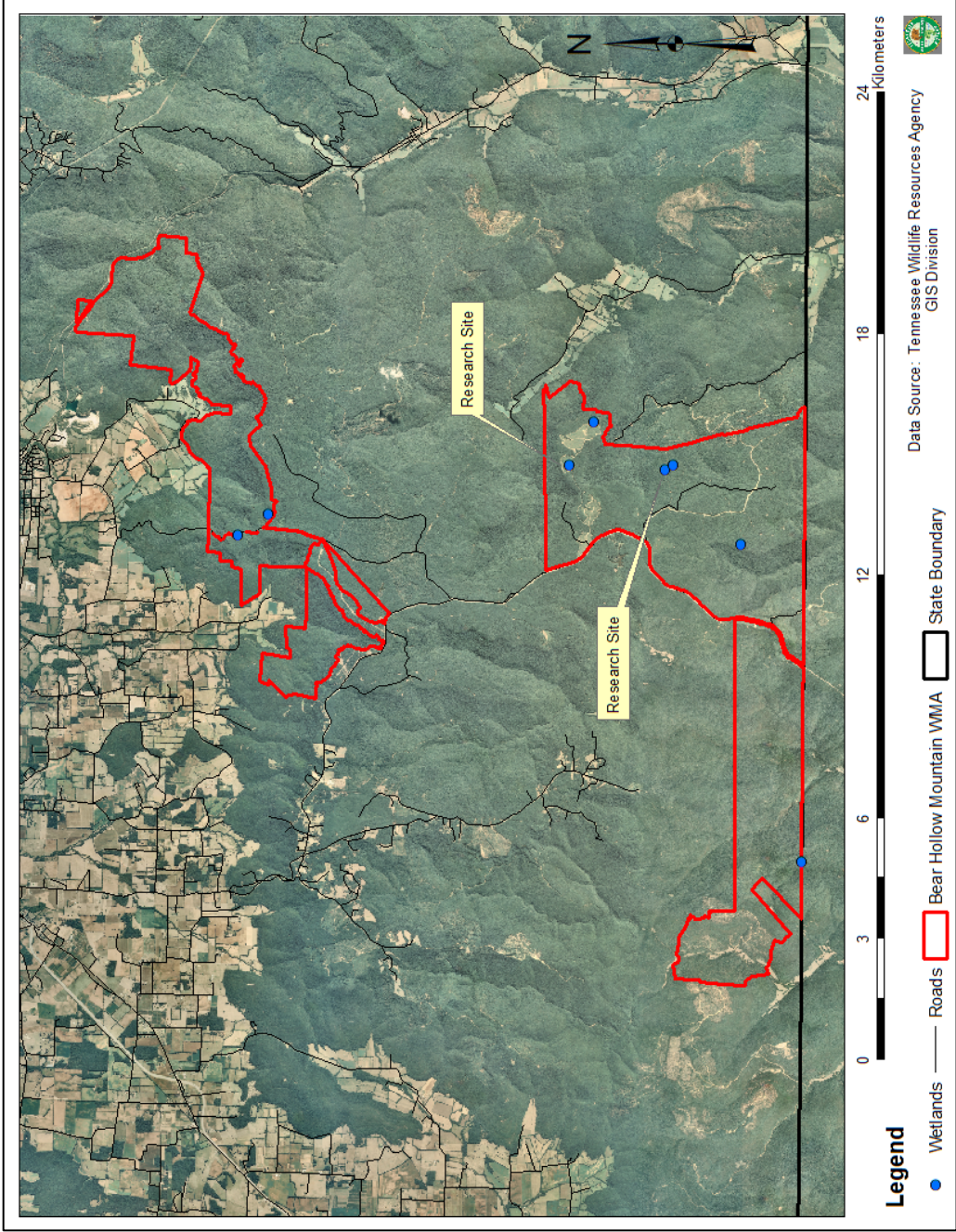


Figure 3. Locations of wetlands on Bear Hollow Mountain WMA as indicated by National Wetland Inventory data.

low amounts of plant nutrients and organic matter.

A closed canopy oak-hickory forest with a relatively open understory surrounded wetland 26007. Plants commonly found in the understory include Greenbriar (*Smilax sp.*), Sassafras (*Sassafras albidum*), Dogwood (*Cornus florida*), and other hardwoods. No overstory existed above the wetland, the dominant plant species within the wetland was Buttonbush (*Cephalanthus occidentalis*), and the wetland margins contained Greenbriar and sedges (*Carex sp.*) (Fig. 4). A road, primarily used for agency access, ran parallel to the eastern portion of the site.

Wetland 26031 was located within a clearcut, which occurred approximately 10 to 14 years ago (Fig. 5). Habitat immediately surrounding this wetland was successional, typical of years following even-age timber harvests. Habitat within the clearcut was composed of numerous species of young hardwoods, blackberry (*Rubus fruticosus*), Broomsedge (*Andropogon virginicus*) and other species of grasses and forbes. A closed canopy oak hickory forest, similar in composition to that surrounding site 26007, surrounded both wetland 26031 and the clearcut.

The forest immediately surrounding the wetland was removed between 1997 and 2005. TWRA acquired the WMA during 2005. The approximate age of the clearcut was ascertained from the metadata associated with digital orthophoto quadrangle imagery taken for Franklin County, Tennessee. Foresters apparently applied the recommended best management practices (BMPs) to wetland 26031, which resulted in approximately 15 m of closed canopy forest being left in the immediate area surrounding the wetland. This buffer of remaining forest adds to the closed canopy already present at the wetland.



Figure 4. The habitat of site 26007.



Figure 5. The habitat of site 26031.

The closed canopy of wetland 26031 is composed primarily of various oak species and Red Maple (*Acer rubrum*). Since acquiring Bear Hollow Mountain WMA, TWRA began slowly converting the existing clearcuts from shrub/scrub habitat to grassland habitat. The conversion process includes the clearcut surrounding wetland 26031. Habitat management began in the winter of 2006 with the implementation of prescribed fire. Recently, heavy equipment was used to remove the undesired habitat, to speed the conversion process, and to create firebreaks and open areas. Figure 6 illustrates the progression of work at this particular wetland.

Throughout the study, bird species observed or heard were recorded (Appendix 1). The presence of specific species illustrates the differences in habitats surrounding wetlands 26007 and 26031. Birds typical of a closed canopy forest found surrounding wetland 26007 include: Black-throated Green Warbler (*Setophaga virens*), Northern Parula (*Setophaga americana*), and Scarlet Tanager (*Piranga olivacea*). The scrub/shrub habitat surrounding wetland 26031 is illustrated by species such as White-eyed Vireo (*Vireo griseus*), Blue-winged Warbler (*Vermivora cyanoptera*), Prairie Warbler (*Setophaga discolor*), Yellow-breasted Chat (*Icteria virens*), and Field Sparrow (*Spizella pusilla*). However, forest obligate bird species such as Black-and-White Warbler (*Mniotilta varia*), Eastern Wood-pewee (*Contopus virens*), Blue-gray Gnatcatcher (*Poliophtila caerulea*) and White-breasted Nuthatch (*Sitta carolinensis*) were observed or heard in the closed canopy forest above site 26031.

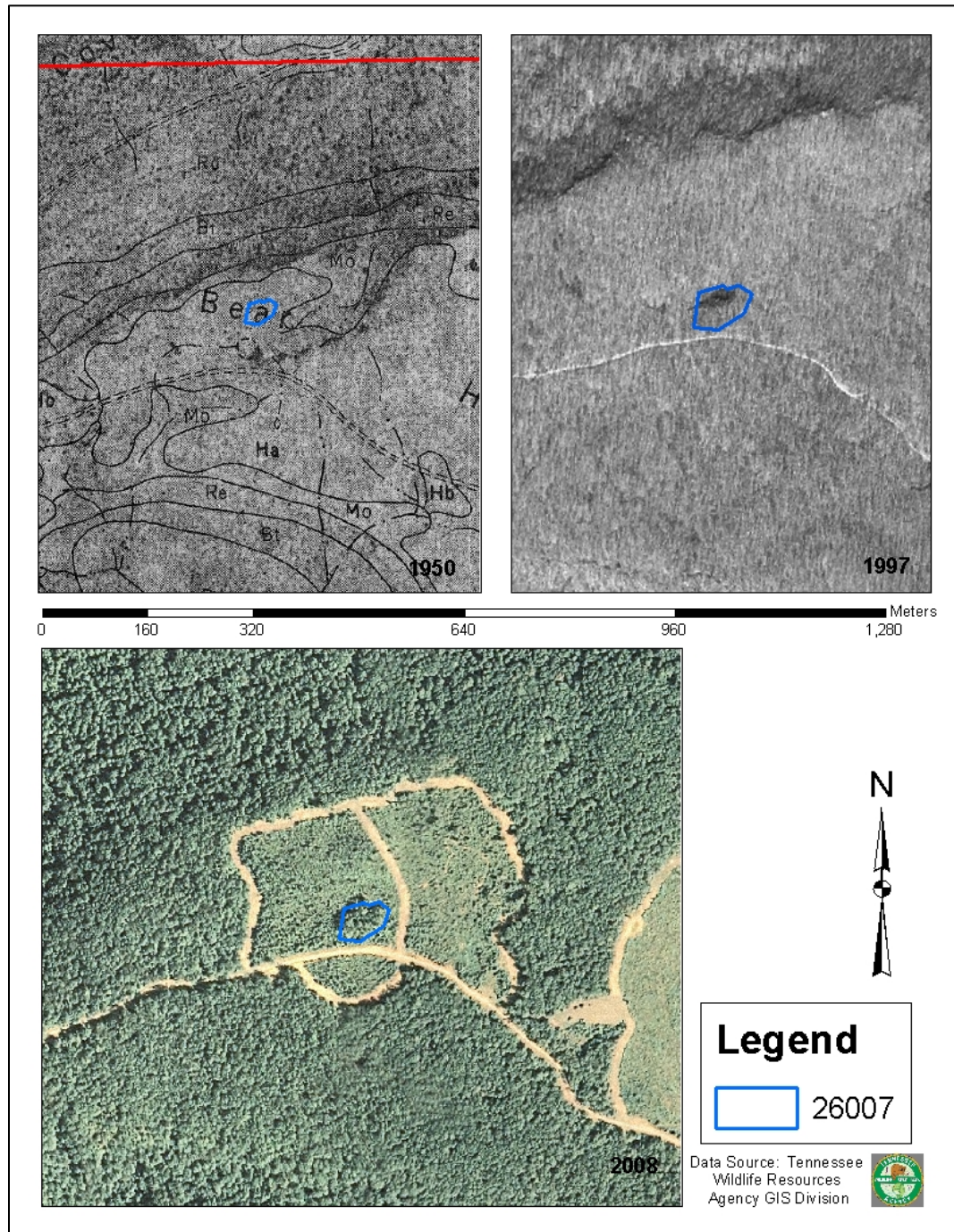


Figure 6. Historic and current status of the upland terrestrial habitat surrounding site 26031.

Chapter 1: Demographics

Implications of habitat management cannot be fully understood unless the characteristics of animal populations inhabiting a given area are known. The main cause of amphibian population declines worldwide is habitat destruction (Sala *et al.* 2000). Habitat fragmentation affects demography by disrupting the distributions of regional and metapopulation processes and increasing extinction rates by increasing distance between populations (Gardner 2001). Small, isolated wetlands typically harbor small amphibian populations and the amount of contact or interactions of populations among these isolated wetlands is small (Mullin and Klueh 2009). Fragmentation of wetlands can have deleterious effects on populations, and can lead to alteration of population demographics, influencing the exchange of genetic material (Lacey 1992).

Populations in a natural setting, not experiencing effects of active habitat management, are susceptible to extinction associated with abiotic factors. Mullin and Klueh (2005) modeled the effects of a short hydroperiod to a population of Jefferson's Salamander (*Ambystoma jeffersonianum*) in Illinois. The model indicated population extirpation was likely because of decreases in annual recruitment brought on by short hydroperiods. Alterations of a habitat can affect hydroperiods, and thereby impact herpetofauna. Changes in the landscape have been linked to changes in the composition of amphibian assemblages (Gray *et al.* 2004). Squamate assemblages are also affected; for example, Hokit and Branch (2003) determined that habitat patch size strongly affects demographics of scrub lizards (*Sceloporus woodi*).

Small wetlands have been shown to harbor high levels of biodiversity (Russell *et al.* 2002); consequently, management of habitat surrounding wetlands can have negative, long-term effects on amphibians. Brodman (2010) discovered that forest dwelling, pond-breeding amphibians took an average of 4.6 years to recover following prescribed burns and recommended decreasing springtime prescribed burn frequency. Compounding the effects of abiotic factors, habitat management can have severe consequences on amphibian populations, especially when decisions are made without the complete understanding of the fauna inhabiting a given area.

Methods

Each wetland was completely encircled with polypropylene fencing material (silt fence) (Fig. 7). Wooden stakes were pre-fastened to the material. The average distance the fence was placed from the water edge was 8.39 m at site 26007 and 4.74 m at site 26031. This distance varied because of the amount of vegetative growth and the predicted high water level at each site. To prevent animals from burrowing underneath the fence, the material was buried approximately 15 cm below the surface (Gibbons and Bennett 1974; Semlitsch 1985; Stenhouse 1985; Palis 2006; Wyckoff 2006). Plastic buckets, 18.9 L, were buried in pairs, one on each side of the fence, spaced approximately 10 m apart (Semlitsch and Pechmann 1985; Dodd 1991; Arntzen 1995; Jenkins 2006 *et al.*; Torrence 2007). All pitfalls were buried flush with the soil surface and placed as close to the fence as possible. To minimize ground water from forcing the traps out of the ground, holes were drilled in the bottom of each pitfall. A small amount



Figure 7. An example of the drift fence and pitfalls used at each site.

of detritus and a wooden block were placed inside each pitfall to reduce mortality associated with desiccation and drowning. Previous studies employed the use of dampened sponges to reduce desiccation (Stenhouse 1985; Jenkins *et al.* 2006; Torrence 2007). In lieu of sponges, detritus was used due to the likelihood of capturing small mammals. While in pitfalls, small mammals destroy sponges, reducing the effectiveness of the technique.

Predator exclusion devices (PEDs) were constructed to minimize the amount of predation associated with this type of study (Dodd 2010). Plastic lids, typically used to close pitfalls, were attached to wooden lumber using wood screws. The wooden lumber was cut to a length of .05 m longer than the depth of the pitfall. The constructed height resulted in a narrow gap between the top of the PED and the pitfall itself, allowing target animals to be captured. Holes were drilled in the top of each PED to allow precipitation into each pitfall, maintaining moisture regimes in traps. PEDs effectively minimized predation, while providing shade to captured animals. Shading of traps is a recommended technique used to minimize mortality (Enge 2001).

To completely assess the demographics and migratory patterns at each site, pitfalls remained opened throughout the duration of the study (site 26007 – 07 December 2007 to 4 May 2010; site 26031 – 11 December 2007 to 4 May 2010). Frequency of trap checks was dependent on weather conditions and the likelihood of migrations of animals. To decrease mortality, trap checks occurred daily during expected amphibian movements (Enge 2001), unless weather was unsuitable for movements. Outside of expected amphibian movements, pitfalls were checked every one to three days.

Box-style funnel traps (Gerald *et al.* 2006) were used to capture larger snake species because of the decreased effectiveness of pitfalls in inventorying these species. Greenberg *et al.* (1994) and Corn (1994) recommended using a combination of both funnel traps and pitfalls to determine species richness of an area. A box-style funnel trap is more effective in capturing larger snakes as compared to single- and double-ended funnel traps (Kjoss and Litvaitis 2001). The design of box-style funnel traps used was modified from Burgdorf *et al.* (2005). All traps were constructed to a dimension of 0.61 m X 0.61 m X 0.38 m using Advantech for the bottom and top. Pressure treated lumber was used to construct a frame to which aluminum wire screen mesh was attached. Funnels were constructed of hardware cloth, paired and placed on opposite sides of the box. The box was divided into two halves with lumber and the aluminum screen wire mesh. This design allowed me to determine whether animals captured were immigrating or emigrating from each site. Silt fence material was placed up to each trap and all gaps between were filled with hardware cloth. Wings, used to direct animals into the trap, were constructed of unused silt fencing material attached to survey stakes and placed at each funnel opening outward at a forty-five degree angle from the trap. Small empty bottles were placed into each funnel, effectively blocking the entrance and eliminating captures during months when data was not collected. To decrease mortality associated with the technique (Dodd 2010), two small plastic containers filled with water and two dampened sponges were placed in each box-style funnel trap (one on each side).

Data were collected using funnel traps during three time periods throughout the study. Trapping dates for 26007 were 07 December 2007 to 17 December 2007, 13 March 2008 to 13 July 2008, and 20 July 2008 to 08 October 2008. Trapping dates for

26031 were 11 December 2007 to 17 December 2007, 13 March 2008 to 13 July 2008, and 20 July 2008 to 08 October 2008. Box-style funnel traps were not used during the 2009 – 2010 field season because of a lack of field technicians. Although box traps were closed at various times during the study, box traps were checked during each site visit because of the potential of animals being incidentally captured with this technique throughout the study. Data was collected from all inadvertent captures, but data was not included in any catch per unit effort calculations.

Each pitfall was assigned a trap identification number (ID) to aid with classification of the migration status of all animals captured. All traps located on the outside of the drift fence were identified with odd numbers, and traps located on the inside of the fence were identified with even numbers. As a result, captures with odd number trap ID's were individuals immigrating into the sites; whereas, individuals with even trap ID's were emigrating from the sites. This system aided with organization of data into immigration and emigration groups.

The identification of all animals captured was made to species. All species deemed greatest conservation need (GCN) by the Tennessee Wildlife Resources Agency were weighed and measured. If possible, the sex of animals captured was determined. Using the appropriate Pesola scales, weights were recorded to the nearest one-half gram. Snout vent lengths and total lengths were determined using dial calipers. All captures were released on the side of the fence opposite of capture.

Individual weights of ambystomatid salamanders were made when manpower allowed. When manpower was lacking, group weights were recorded. Captured ambystomatid salamanders were separated by species and group weights of each sex

were recorded. Although individual weights were preferred, group weights allowed for the collection of total biomass to be recorded by sex. TWRA personnel collected data between December 2007 and the fall of 2009. MTSU personnel began collection of data during the fall 2009 with the approval of all protocols (11-006) by the Middle Tennessee State University Office of Compliance Institutional Animal Care and Use Committee.

Chi-square tests were conducted to determine if the proportion of males and females for each species of ambystomatid salamanders were equal at each site. Using recorded snout-vent lengths, *t*-tests were used to test the hypotheses that SVL were the same between the sexes of ambystomatid species at each site and between sites. All *t*-tests were conducted using PASW Statistics 18.0 (SPSS Inc., Chicago, Ill.). Contingency tables were used to test the hypothesis the number of juveniles captured were the same between years and sites. Contingency table calculations were made using Microsoft Excel (Microsoft Corporation ® 2008) and GraphPad Software, available online (<http://graphpad.com/quickcalcs/contingency1.cfm>, accessed 19 October 2013).

Results

Between 2007 and 2010, consecutive trapping at both sites combined produced 38,063 captures. Fifty-five species of amphibian, reptile, and small mammal were captured during the study, 47 species at site 26007 and 41 species at site 26031. Thirty-six species of amphibians and reptiles were captured at site 26007 and thirty-one species of amphibians and reptiles were captured at site 26031.

Appendix 2 contains tables indicating species, conservation status, and capture totals for each site. Nine GCN species were captured, eight species at 26007 and five at

26031. GCN species captured include: Four-toed Salamander, (*Hemidactylium scutatum*), Barking Treefrog (*Hyla gratiosa*), Hognosed Snake (*Heterodon platirhinos*), Timber Rattlesnake (*Crotalus horridus*), Eastern Box Turtle (*Terrapene carolina*), Eastern Woodrat (*Neotoma floridana*), Southeastern Shrew (*Sorex longirostris*), Pygmy Shrew (*Sorex hoyi*), and Golden Mouse (*Ochrotomys nuttalli*).

Ambystomatid Salamanders

Four species of ambystomatid salamanders were captured at each site: *Ambystoma opacum*, *Ambystoma maculatum*, *Ambystoma talpoideum*, and *Ambystoma tigrinum*. Ambystomatids accounted for 80% of the overall capture total, 80% and 78% of the capture total for sites 26007 and 26031, respectively. *Ambystoma maculatum* was the most abundant species captured at site 26007, followed by *A. opacum*. At site 26031, *A. opacum* was more abundant than *A. maculatum*. Only seven total captures of *A. tigrinum* occurred (four at site 26007 and 3 at 26031), and because of this, no analyses of this species were conducted.

Site 26007

This study began after the immigration of *A. opacum* to each site had occurred; because of this, analyses of *A. opacum* data were limited to Years 2 and 3. Average SVL differed significantly between males and females, $t_{(79)} = 2.843$, $p = 0.006$, with females smaller than males (Female SVL $\bar{x} = 61.42$, SD = 5.86, Male SVL $\bar{x} = 64.71$, SD = 4.51). Male *A. opacum* outnumbered females over the course of the study (1:1.199, $\chi^2 (1, N = 2,983) = 24.62$, $p < 0.0001$), during Year 2 (1:1.112, $\chi^2 (1, N = 2,720) = 7.62$, $p < 0.01$), and during Year 3 (1:2.868, $\chi^2 (1, N = 263) = 61.32$, $p < 0.0001$).

Female *A. maculatum* (SVL \bar{x} = 104.08, SD = 9.59) were larger than males (SVL \bar{x} = 91.76, SD = 6.90), $t_{(324)} = -12.728$, $p < 0.0001$. Male *A. maculatum* outnumbered females overall (2.774:1, χ^2 (1, $N = 5,590$) = 1,235.48, $p < 0.0001$), during Year 1 (3.475:1, χ^2 (1, $N = 3,540$) = 1,082.90, $p < 0.0001$), during Year 2 (1.891:1, χ^2 (1, $N = 928$) = 88.14, $p < 0.0001$), and during Year 3 (2.041:1, χ^2 (1, $N = 1,122$) = 131.42, $p < 0.0001$).

More male *A. talpoideum* were captured than females during the three years (1.705:1, χ^2 (1, $N = 1,761$) = 119.64, $p < 0.0001$), during Year 1 (1.877:1, χ^2 (1, $N = 1,148$) = 106.70, $p < 0.0001$), during Year 2 (1.295:1, χ^2 (1, $N = 404$) = 6.70, $p < 0.01$), and during Year 3 (1.750:1, χ^2 (1, $N = 209$) = 15.54, $p < 0.0001$). Male SVL (SVL \bar{x} = 61.37, SD = 4.31) was significantly larger than female SVL (SVL \bar{x} = 58.69, SD = 4.20), $t_{(319)} = 4.280$, $p < 0.0001$.

Site 26031

Data analyses for *A. opacum* were limited to Years 2 and 3. Throughout the duration of the study, female *A. opacum* were more numerous than males (3.913:1, χ^2 (1, $N = 506$) = 177.86, $p < 0.0001$). The sex ratio during Year 2 was female biased (4.686:1, χ^2 (1, $N = 398$) = 167.24, $p < 0.0001$), and the smallest during Year 3 (2.273:1, χ^2 (1, $N = 108$) = 16.34, $p < 0.0001$). Male SVL (SVL \bar{x} = 63.47, SD = 4.64) was significantly greater than female SVL (SVL \bar{x} = 57.80, SD = 5.99), $t_{(36)} = 3.10$, $p = 0.004$.

Female *A. maculatum* (SVL \bar{x} = 100.07, SD = 5.87) were significantly larger than males (SVL \bar{x} = 88.56, SD = 5.62), $t_{(84)} = -8.309$, $p < 0.0001$. Sex ratios were skewed toward males throughout the study (1.249:1, χ^2 (1, $N = 940$) = 11.50, $p < 0.0001$).

During Year 1, males were more numerous than females (1.281:1, χ^2 (1, $N = 584$) = 8.88, $p < 0.01$), and the sex ratio was the largest during Year 2, (1.476:1, χ^2 (1, $N = 156$) = 5.76, $p < 0.02$). Although the ratio decreased during Year 3, males still outnumbered females, but only slightly (1.020:1, χ^2 (1, $N = 200$) = 0.02, $p > 0.20$).

The SVL of female *A. talpoideum* (SVL \bar{x} = 57.99, SD = 3.87) was significantly smaller than that of males (SVL \bar{x} = 59.46, SD = 4.19), $t_{(182)} = 2.435$, $p = 0.016$).

Females outnumbered males throughout the study (1.269:1, χ^2 (1, $N = 595$) = 8.13, $p < 0.01$), and during Year 1 (1.328:1, χ^2 (1, $N = 419$) = 8.3, $p < 0.01$). However, the number of females did not differ from males during either Year 2 (1:119:1, χ^2 (1, $N = 89$) = 0.028, $p > 0.20$) or Year 3 (1.158:1, χ^2 (1, $N = 82$) = 0.440, $p > 0.20$).

Between Sites

Female *A. opacum* at site 26007 (SVL \bar{x} = 61.42, SD = 5.86) were significantly larger than those at site 26031 (SVL \bar{x} = 57.80, SD = 5.99), $t_{(52)} = 2.221$, $p = 0.031$.

Female *A. talpoideum* at site 26007 (SVL \bar{x} = 58.69, SD = 4.20) did not differ significantly in size from females at site 26031 (SVL \bar{x} = 57.99, SD = 3.87), $t_{(133)} = 1.01$, $p = 0.315$. Female *A. maculatum* at site 26007 (SVL \bar{x} = 104.08, SD = 9.59) did not differ significantly in size from females at site 26031 (SVL \bar{x} = 100.07, SD = 5.87), $t_{(107)} = 1.908$, $p = 0.059$.

Male *A. opacum* at site 26007 (SVL \bar{x} = 64.71, SD = 4.51) did not differ in size from males at site 26031 (SVL \bar{x} = 63.47, SD = 4.64), $t_{(63)} = 0.931$, $p = 0.355$. SVL of male *A. talpoideum* from site 26007 (SVL \bar{x} = 61.37, SD = 4.31) differed significantly from those at site 26031 (SVL \bar{x} = 59.46, SD = 4.19) $t_{(368)} = 3.888$, $p < 0.0001$.

Ambystoma maculatum males from site 26007 (SVL \bar{x} = 91.76, SD = 6.90) were significantly larger than those from site 26031 (SVL \bar{x} = 88.56, SD = 5.62), $t_{(301)} = 3.395$, $p = 0.001$.

Juveniles

Analysis of juvenile data was limited to Years 1 and 2 because data collection during Year 3 did not completely census juvenile movement. Juvenile recruitment for each of the ambystomatid species was stochastic between species, sites, and years (Table 1). The number of juveniles captured for each species was highest during the first year and decreased during the second year.

The number of captures of juvenile *A. opacum* was higher during the first year at site 26007 than the second, $\chi^2 (1, N = 6,782) = 172.9, p < 0.0001$. *A. maculatum* juvenile captures at site 26007 was greatest during Year 1 than Year 2, $\chi^2 (1, N = 680) = 21.6, p < 0.0001$. The number of captures of *A. talpoideum* was higher during the first year at site 26007 than the second year, $\chi^2 (1, N = 1,184) = 114.4, p < 0.0001$ (Table 1).

Discussion

The diversity of amphibians and reptiles at the wetlands studied at Bear Hollow Mountain WMA is similar to or exceeds that of other studies in similar habitats (Russell *et al.* 1999; Wyckoff 2006; Davenport and Scott 2009). The herpetofaunal diversity indicates the significance of each wetland within the landscape. Current management of Bear Hollow Mountain WMA is focused on converting existing even-aged silvicultural treatments to grassland habitats. Because of this, appropriate habitat surrounding site

	Number of Captures			Total	X^2_s	df	<i>p</i> value
	Year	26007	26031				
<i>A. opacum</i>	1	3,727 (3,523.09)	1,762 (1,965.91)	5,489	172.9	1	< 0.0001
	2	626 (829.91)	667 (463.09)	1,293			
		4,353	2,429	6,782			
<i>A. maculatum</i>	1	522 (510.77)	120 (131.23)	642	21.6	1	< 0.0001
	2	19 (30.23)	19 (7.77)	38			
		541	139	680			
<i>A. talpoideum</i>	1	585 (653.68)	352 (283.32)	937	114.4	1	< 0.0001
	2	241 (172.32)	6 (74.68)	247			
		826	358	1,184			

Table 1. Observed and expected (parentheses) capture totals for ambystomatid salamanders at sites 26007 and 26031.

26007 will be protected into the foreseeable future. Proposed land management to this area should be reviewed and considerations must be given to affects any habitat alteration may have on herpetofaunal diversity.

Although the diversity of each wetland is significant, the occurrence of early successional habitat surrounding site 26031 has created niches not present at site 26007, allowing for differences in species diversity. These differences are reflected by species of breeding birds in the vicinity of site 26031, as well as the occurrence of reptile species, such as *Heterodon platirhinos*. The lack of *Plethodon glutinosus* from site 26031 may also be indicative of habitat differences between the two wetlands. This species typically inhabits moist microhabitats of the forest floor (Petranka 1998).

Sex ratios observed at each site were similar to those reported elsewhere for *A. opacum* (Stenhouse 1987), *A. maculatum* (Husting 1965, Whitford and Vinegar 1966, Stenhouse 1987, Phillips and Sexton 1989, Homan *et al.* 2007), and *A. talpoideum* (Semlitsch 1985, Raymond and Hardy 1990, Semlitsch *et al.* 1993). Sex ratios varied for each species annually and between sites. Variation in sex ratios between years may be the result of biennial mating patterns of females (Husting 1965). In the case of *A. talpoideum*, sex ratios approached 1:1 in Years 2 and 3 at site 26031, but capture totals were the lowest during these years. Decreases in the sex ratios for this species correspond to their abundance at this site.

The only species with sex ratios skewed toward females was *A. opacum*. Ratios of females to males were greatest at site 26031. Differing from other ambystomatids present at each site, *A. opacum* breeds on land. This species oviposits in terrestrial nests prior to the filling of wetlands; thus, wetlands are not a requirement for mating, which

can occur prior to entry into a wetland. Krenz and Scott (1994) observed female *A. opacum* captured away from nesting sites deposit fertilized eggs in a laboratory setting. The ability of males to inseminate females prior to entry of wetlands decreases their presence at the wetlands and results in sex ratios skewed towards females.

Upland habitat preferences of wetland breeding ambystomatid salamanders are typically characterized as mature, deciduous or floodplain forests (Petranka 1998). The creation of grassland habitat around a wetland leads to forest fragmentation and creates movement barriers for salamanders, decreasing their reproductive ability. Migrations of ambystomatids may be limited by habitat features within the landscape, including agriculture fields, grasslands, permanent ponds, and residential areas (Madison and Farrand 1998; Faccio 2003; Nussbaum 2005). However, studies suggest fragmentation within the landscape may not disrupt the breeding behavior of these salamanders as previously thought. *Ambystoma maculatum* has been observed migrating through open habitats (Montieth and Paton 2006; McDonough and Paton 2007), and *A. tigrinum* will use agricultural and semi-opened habitats (Steen *et al.* 2006), indicating these species will use wetlands within a fragmented landscape. Given the abundance and presence of four species of ambystomatid salamanders at site 26031, current habitat management appears not to negatively affect the migratory behavior of these species.

Given the abundance of adult salamanders captured throughout the study, the lack of recruitment for all ambystomatids is concerning. Although recruitment was considerably higher during Year 1 and capture totals were significantly different between years, few juvenile *A. maculatum* or *A. talpoideum* were captured. Survivorship of larval ambystomatids at each site is density-dependent, influenced by mortality of developing

embryos and larvae, and inter- and intra-specific competition (Shoop 1974). *Ambystoma maculatum* and *A. talpoideum* survivorship was potentially reduced, in part, by the high numbers of *A. opacum* larvae present at each site. The eggs of *A. opacum* are laid months earlier than those of *A. talpoideum* and *A. maculatum*. This breeding strategy allows *A. opacum* larvae to develop more quickly, exploit resources sooner, and metamorphose prior to other species of ambystomatid.

The lack of recruitment at each site during each year may also be attributed to a lack of eggs deposited each year by ambystomatids. Recruitment decreased each year, as did the presence of adult salamanders at each site. Although it is easy to assume recruitment is dependent upon the number of eggs deposited by adults, Shoop (1974) determined variation in survivorship between years is not dependent solely on the number of eggs deposited. The duration the wetland contains water influences the survivorship of larvae. Shorter hydrologic cycles may decrease survivorship, reflecting the low number of juveniles captured each year.

Although it does not appear that the clearcut and ground disturbance is impacting adult immigration and emigration at site 26031, developing and dispersing juvenile salamanders are most impacted by these habitat modifications (Rothermel and Semlitsch 2002; Rothermel and Luhring 2005; Rothermel and Semlitsch 2006). Successful metamorphosis and dispersal was greatest during the first year and decreased in successive years at each site; however, the number of juveniles exiting site 26031 was significantly lower for all three ambystomatids when compared to site 26007. The lack of juvenile recruitment observed is likely associated with the shortened hydroperiod at 26031 compared to 26007. The drying of a breeding pond determines the success of

juvenile recruitment and if this occurs too soon, catastrophic loss of juvenile amphibians reaching metamorphosis may be observed (Semlitsch 1987; Pechmann *et al.* 1989; Pechmann *et al.* 1991). Although these catastrophic failures may be numerous and indicate declines, juvenile recruitment is episodic (Semlitsch 2002), and may occur periodically allowing for maintenance of the population.

Current habitat management implemented by TWRA at 26031 does not appear to negatively impact the amphibian migration and species diversity. Differences in species diversity between the two sites only indicate the differences in habitat at 26031 and 26007. Despite the lack of impact of habitat management on adult salamanders, TWRA should reevaluate the current habitat management being implemented to the landscape surrounding 26031 because of potential impacts to juvenile recruitment of ambystomatids. Allowing the current open habitat to succeed into forest should decrease impacts from solar exposure and temperature, thereby increasing hydroperiod. Although juvenile recruitment is episodic, the habitat management on Bear Hollow Mountain WMA should seek to improve the quality of the wetlands and terrestrial habitat to increase the success of juvenile recruitment when it is most likely to occur.

Chapter 2: Orientation

Knowing the land-use patterns of amphibians can be critical to determining the appropriate land management and conservation actions to implement. Conservation buffers, similar to those proposed by Semlitsch (1998), are circular in shape and estimate the amount of upland habitat used by pond breeding amphibians. The size of conservation buffers varies among species (Semlitsch and Bodie 2003; Crawford and Semlitsch 2007). Although the use of the recommended buffers seeks to protect a set amount of upland habitat, blanket application of conservation buffers may not incorporate important habitat or features within the landscape. These important areas will only be indicated through site research. For example, numerous studies have been conducted on the land-use patterns of pond-breeding amphibians and indicate migrations to and from breeding sites are typically nonrandom (Kleeberger and Werner 1983; Stenhouse 1985; Phillips and Sexton 1989; Malmgren 2002; Homan *et al.* 2008; Todd *et al.* 2009). Nonrandom movements suggest amphibians might be using specific corridors within the landscape for migration. Sztatecsny and Schabetsberger (2005) detected nonrandom movement of Common Toads (*Bufo bufo*) across alpine habitats, identifying important movement corridors associated with extreme vertical movements of this species. The extreme vertical movements exhibited by *B. bufo* were made to reach high quality habitats away from breeding sites. Conservation buffers that are based on the species movements at lower elevations would not necessarily protect the corridors used to reach these habitats.

Although migrations of pond-breeding amphibians may be random, short-term studies indicate migratory patterns indicative of important habitat features. deMaynadier and Hunter (1999) during a single field season observed juvenile *A. maculatum* select forested upland habitats during emigration. Eastern Long-toed Salamanders (*Ambystoma macrodactylum columbianum*) migrate randomly through habitat surrounding breeding sites (Beneski *et al.* 1986), but data were collected during a single breeding season. Santos *et al.* (2010) determined the movements of a population of Brazilian Red-bellied Toads (*Melanophryniscus cambarensis*) to be directional. Movements between males and females differed, and migrations were constrained to only a portion of the breeding site. Although the upland habitat surrounding the site was homogenous, the movement pattern presumably was in response to a road that fragmented the site. Timm *et al.* (2007b) conducted a long-term study assessing the emigration orientation of juvenile amphibians from multiple wetlands. Although emigrations among years indicated directionality, assessing emigrations across several years showed considerable variation in orientation. This study indicates the need to conduct long-term studies to accurately determine the necessary conservation actions.

To determine if proposed conservation buffers would suffice in protecting the necessary habitat surrounding the research sites on Bear Hollow Mountain WMA, land-use patterns of three species of ambystomatid (*A. opacum*, *A. maculatum*, and *A. talpoideum*) were studied. If migratory patterns by these species are non-random, additional conservation strategies may be necessary to ensure protection of amphibian populations at these breeding sites on the WMA.

Methods

All salamanders were captured according to the methods described earlier (page 19). A hand-held GPS (Garmin model 76CSx) was used to record the location of drift fences, pitfalls and box-style funnel traps at each site. Data were transferred to ESRI ArcMap 9.3 via MN DNR Garmin (Minnesota Department of Natural Resources, St. Paul, MN). Bearings of each pitfall from the center of each site were determined using ESRI ArcView 3.3 (Environmental Systems Research Institute, Redlands, CA). Bearings were then used to determine migratory patterns of ambystomatid salamanders at each site. The orientation of migrating salamanders was tested in Oriana 4.0 (Kovach Computing Services, Wales, UK) using Rao's Spacing Test. This test was used to determine if migration patterns were uniformly distributed across each site or congregated along any measured bearing(s).

A number of assumptions of the measured migrations were used to test uniformity. Immigration to and emigration from each site was assumed to be linear. It was assumed captured salamanders entered the first trap encountered and no traps were bypassed during migrations. The bearing of migration was assumed to be equal to that of capture locations. The bearing of each salamander was recorded and plotted on circular frequency histograms using Oriana 4.0 (*op.cit.*). Rao's Spacing Test was used to determine if migrations of salamanders were uniform. A lack of uniformity indicates movement corridors. Species data were analyzed by (1) immigration of females and males by year, (2) emigration of animals by year, (3) emigration of juveniles by year, and (4) all data were combined across the length of the study for immigrating females and

males, and emigrating juveniles and adults. Emigration analyses were not conducted by sex because ambystomatids typically lose external sexual characteristics post-breeding making identification difficult in the field. First year data for adult *A. opacum* were not analyzed for either site because surveys began following immigrations. Also, juvenile emigration was not analyzed for any species during Year 3 because trapping ceased prior to the conclusion of the movements; however, any juveniles captured prior to the conclusion of trapping were included in study-wide analysis. Because of the lack of data collected for *A. tigrinum*, no analyses were conducted for this species. Mean vectors of movement and Rao's Spacing Test results are summarized in Appendix 3.

Results

26007

Ambystoma opacum

Immigration patterns of male *A. opacum* were not uniform during Year 2 ($U = 352.936, p < 0.01$) and Year 3 ($U = 284.614, p < 0.01$, Fig. 8). Female *A. opacum* immigration lacked uniformity during Year 2 ($U = 353.950, p < 0.01$) and Year 3 ($U = 324.923, p < 0.01$, Fig. 8). Furthermore, adult emigration patterns were not uniform in Year 2 ($U = 345.749, p < 0.01$) and Year 3 ($U = 333.976, p < 0.01$, Fig. 9). Juvenile emigrations were not uniformly distributed during Year 1 ($U = 357.382, p < 0.01$) or Year 2 and ($U = 351.162, p < 0.01$, Fig. 9). Combined immigration of male *A. opacum* and all immigrating *A. opacum* were not uniformly distributed across the site (All Male $U = 353.363, p < 0.01$, All Immigrants $U = 357.21, p < 0.01$, Fig. 10). Combined female immigration, combined juvenile, and adult emigration differed from parity at site

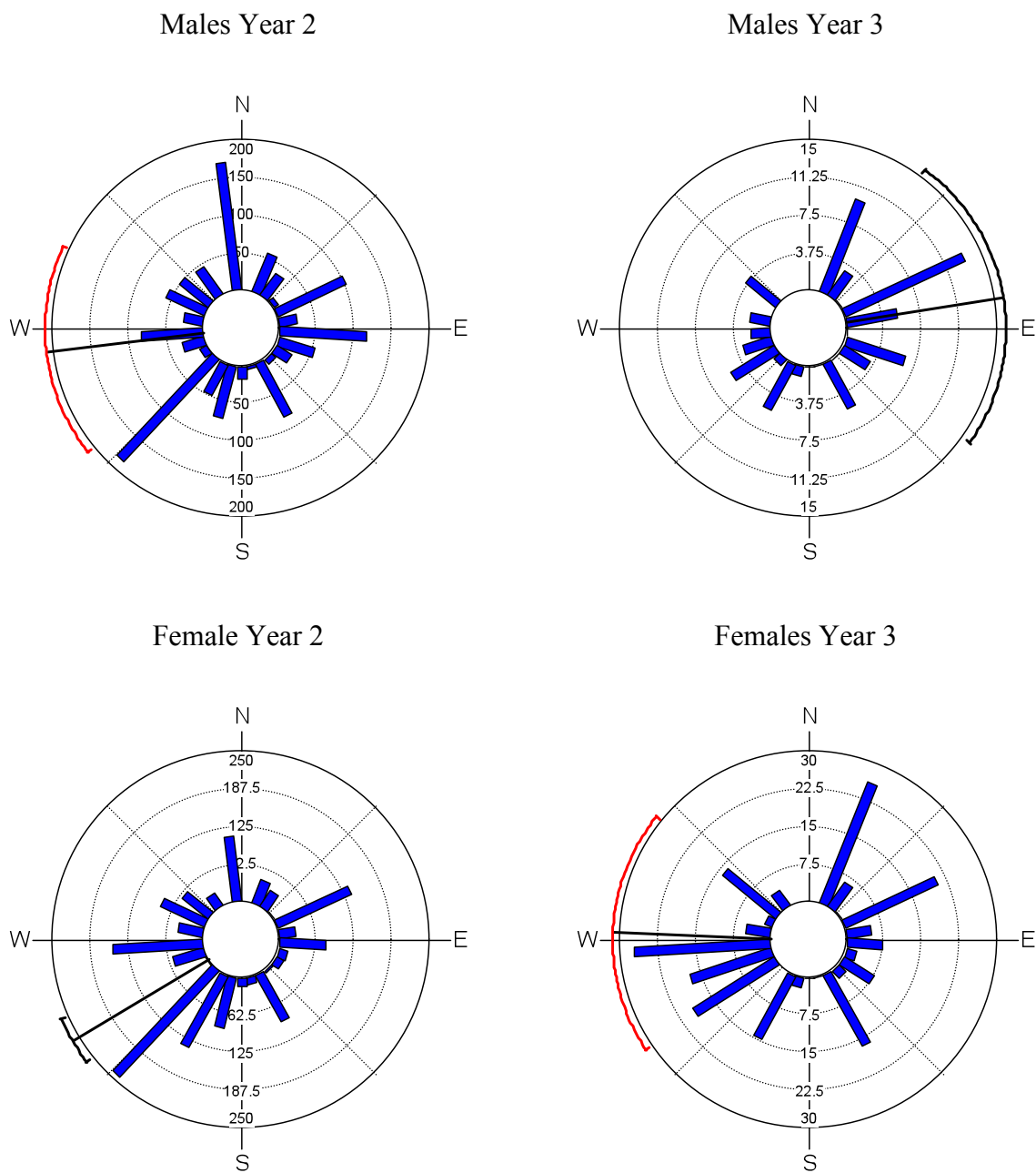


Figure 8. Orientation of adult female and male *A. opacum* at site 26007 during Year 2 and Year 3. The solid black line indicates the mean direction of movement, and 95% confidence intervals. Red confidence intervals may be unreliable due to a lack of concentration.

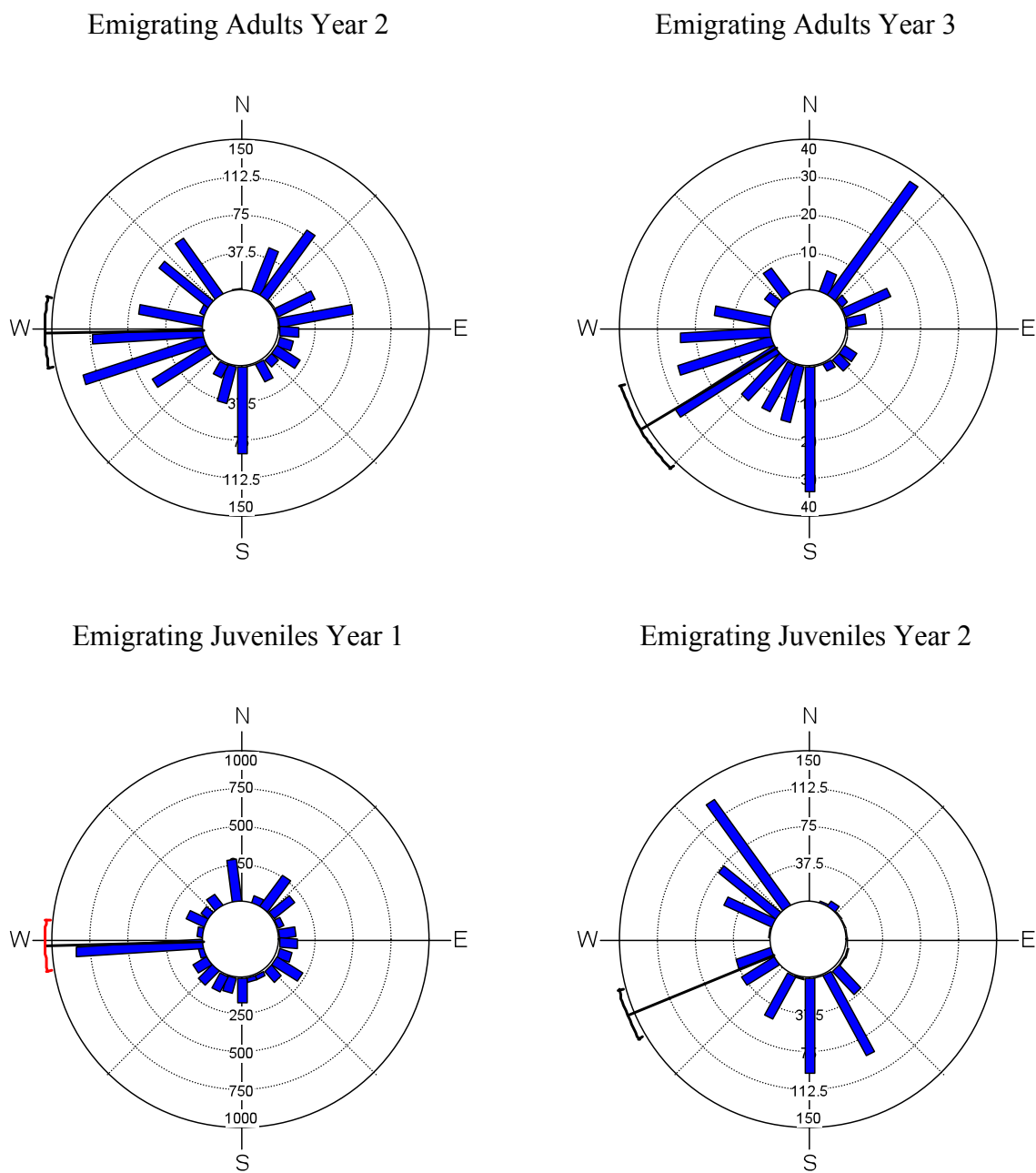


Figure 9. Orientation of emigrating adult and juvenile *A. opacum* at site 26007. The black line indicates the mean direction of movement, and 95% confidence intervals. Red confidence intervals may be unreliable due to a lack of concentration.

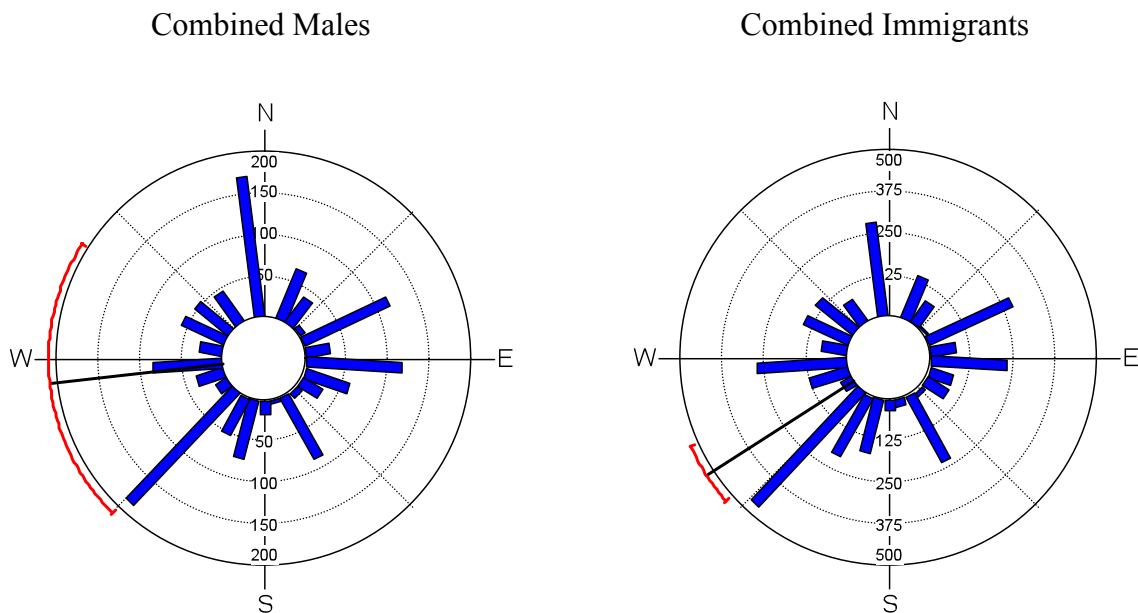


Figure 10. Migratory patterns of combined male and immigrating *A. opacum* at site 26007. The black line indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable due to the lack of concentration

26007 (Female $U = 354.686$, $p < 0.01$, Juvenile $U = 357.712$, $p < 0.01$, Adult Emigrants $U = 352.993$, $p < 0.01$, Fig. 11)

Ambystoma maculatum

Immigrations of male and female *A. maculatum* were not uniformly distributed in any year (Male Year 1 $U = 357.270$, $p < 0.01$, Male Year 2 $U = 347.627$, $p < 0.01$, Male Year 3 $U = 350.438$, $p < 0.01$, Female Year 1 $U = 351.787$, $p < 0.01$, Female Year 2 $U = 338.692$, $p < 0.01$, Female Year 3 $U = 342.439$, $p < 0.01$, Fig. 12). Adult emigrations were not uniformly distributed during any year (Year 1 $U = 352.421$, $p < 0.01$, Year 2 $U = 347.713$, $p < 0.01$, Year 3 $U = 335.676$, $p < 0.01$, Fig. 13). Distributions of juvenile emigrations were not uniform during any year (Year 1 $U = 340.851$, $p < 0.01$, Year 2 $U = 194.700$, $p < 0.01$, Fig. 14). Combined male and female immigrations and all immigrations differed from parity (Combined Male $U = 357.899$, $p < 0.01$, Combined Female $U = 355.132$, $p < 0.01$, All Immigrants $U = 358.441$, $p < 0.01$, Fig. 15), and mean movements were concentrated along easterly vectors. Combined juvenile and adult emigrations were not uniformly distributed across the site (Combined Juveniles $U = 343.364$, $p < 0.01$, Combined Emigrants $U = 357.323$, $p < 0.01$, Fig. 16)

Ambystoma talpoideum

Immigration movements of both male and female *A. talpoideum* lacked uniformity across all years (Male Year 1 $U = 348.465$, $p < 0.01$, Male Year 2 $U = 331.703$, $p < 0.01$, Male Year 3 $U = 310.909$, $p < 0.01$, Female Year 1 $U = 342.857$, $p < 0.01$, Female Year 2 $U = 325.227$, $p < 0.01$, Female Year 3 $U = 284.547$, $p < 0.01$, Fig 17.). Adult emigrations were not uniformly distributed during any year (Year 1 $U =$

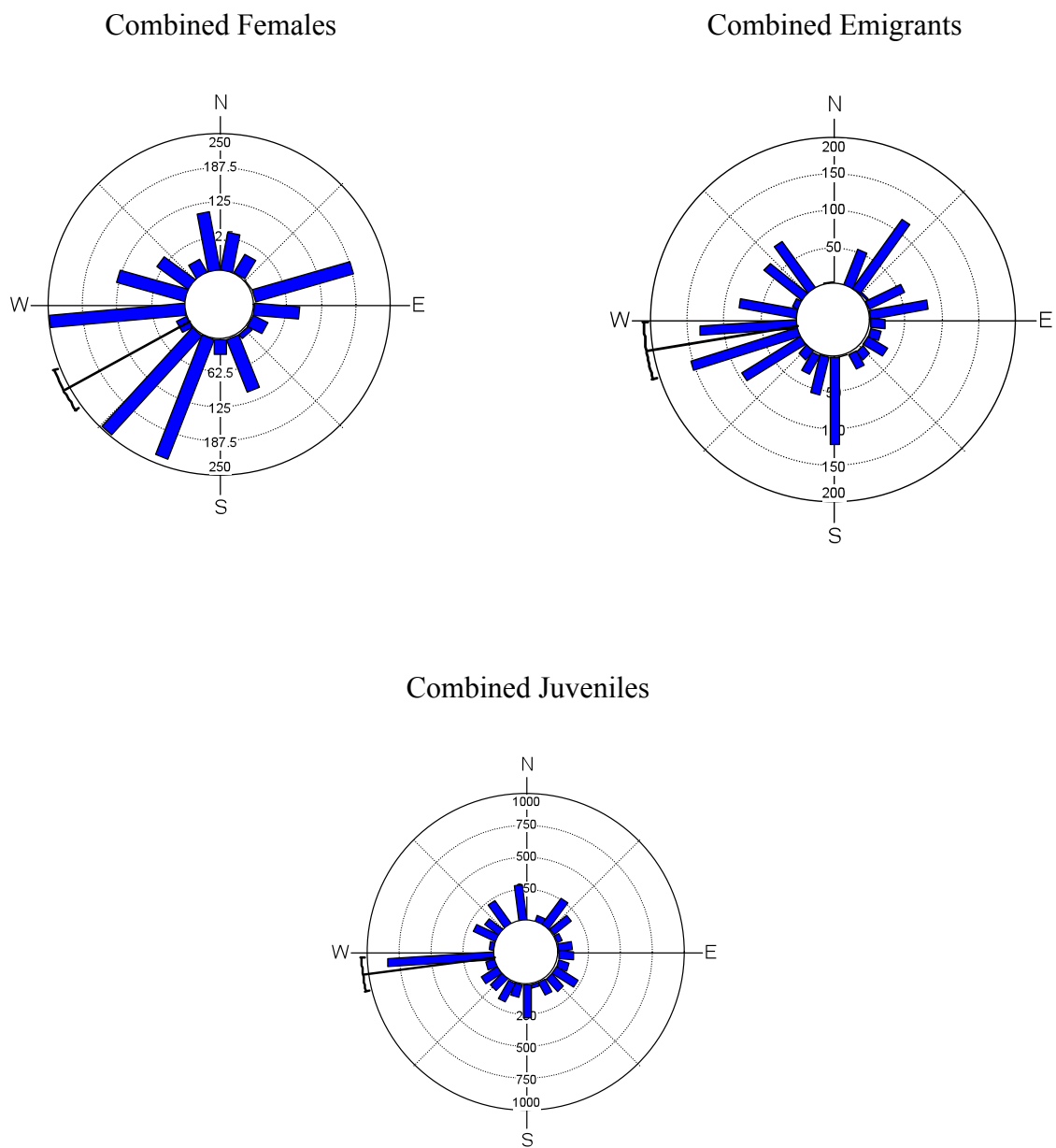


Figure 11. Orientation of combined female immigrants, all emigrants, and juvenile *A. opacum* at site 26007. The black line indicates the mean direction of movement, and 95% confidence intervals.

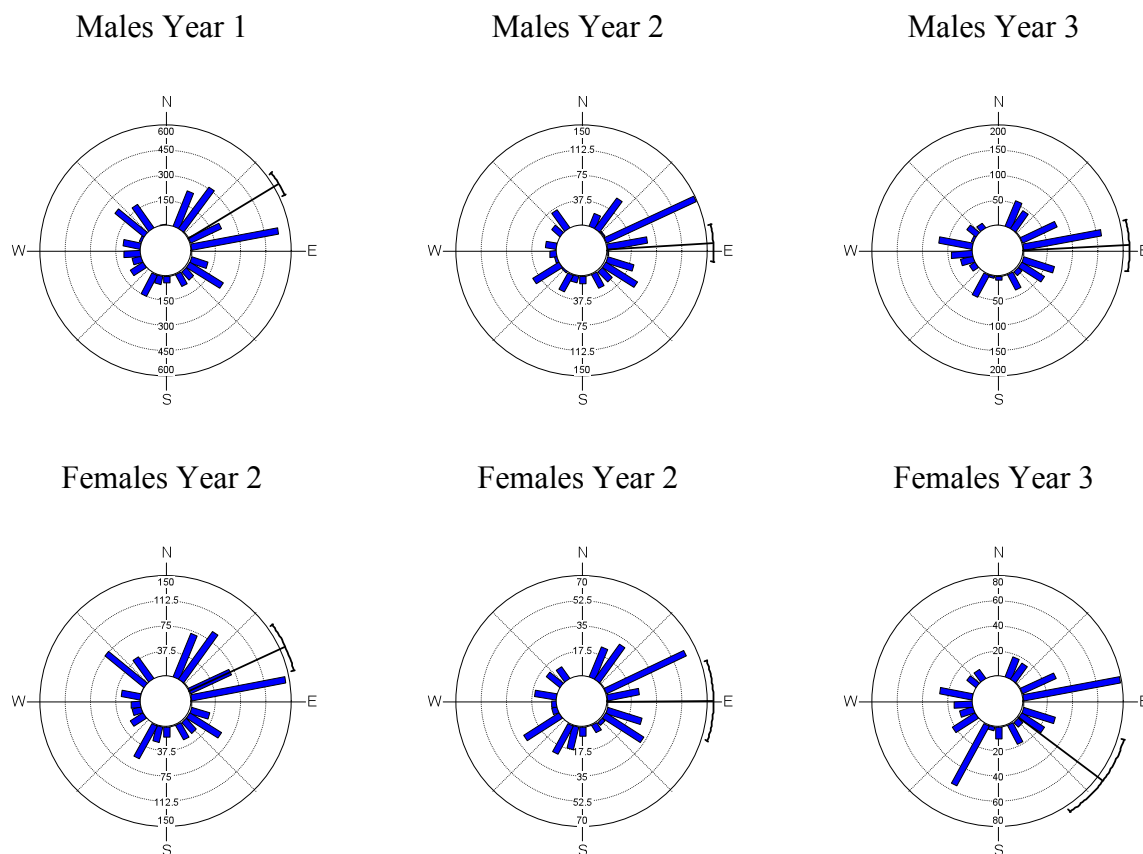


Figure 12. Orientation of immigrating adult male and female *A. maculatum* at site 26007. The black line indicates the mean direction of movement and 95% confidence intervals.

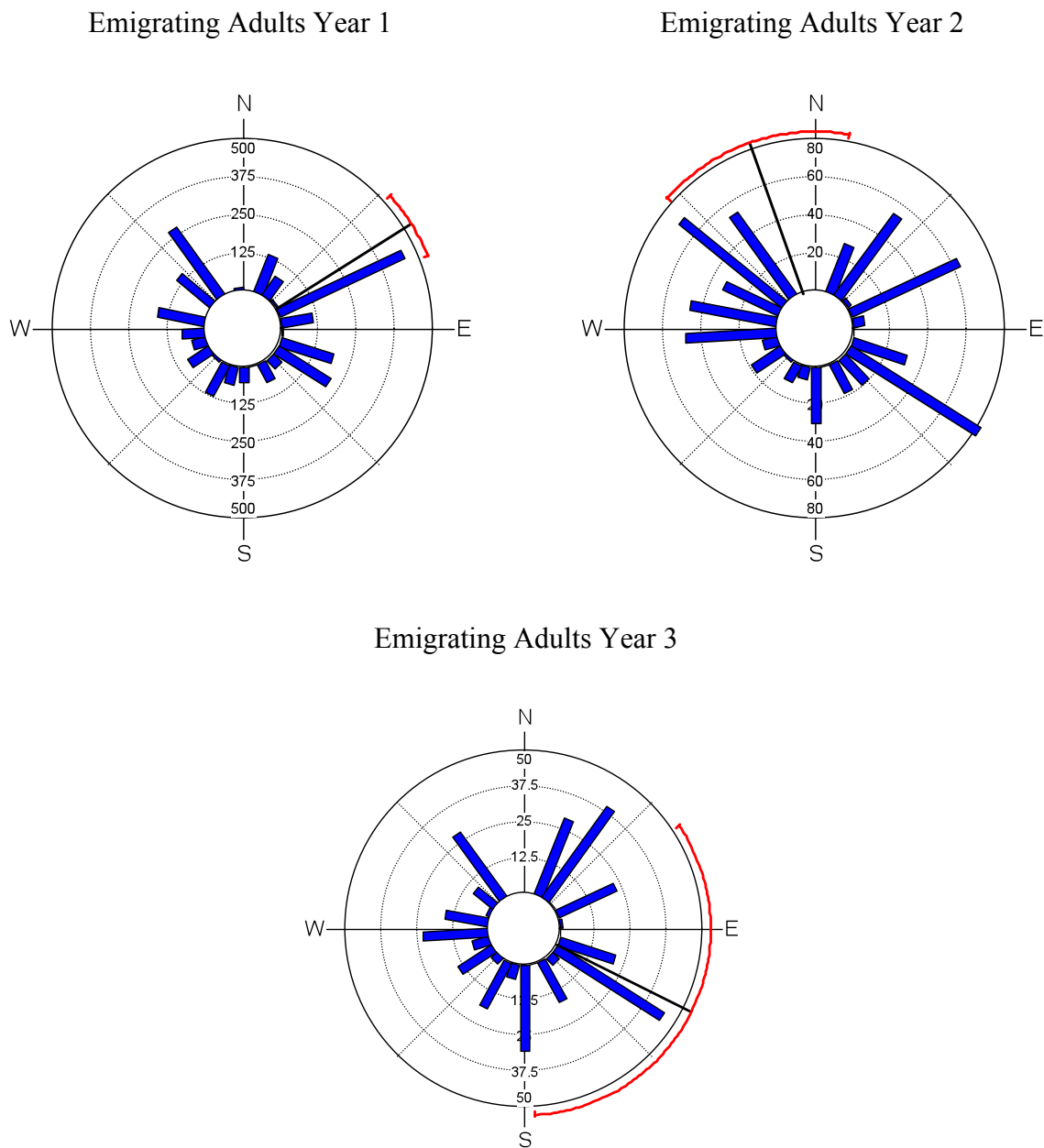


Figure 13. Orientation of emigrating adult *A. maculatum* at site 26007. The black line indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable due to the lack of concentration.

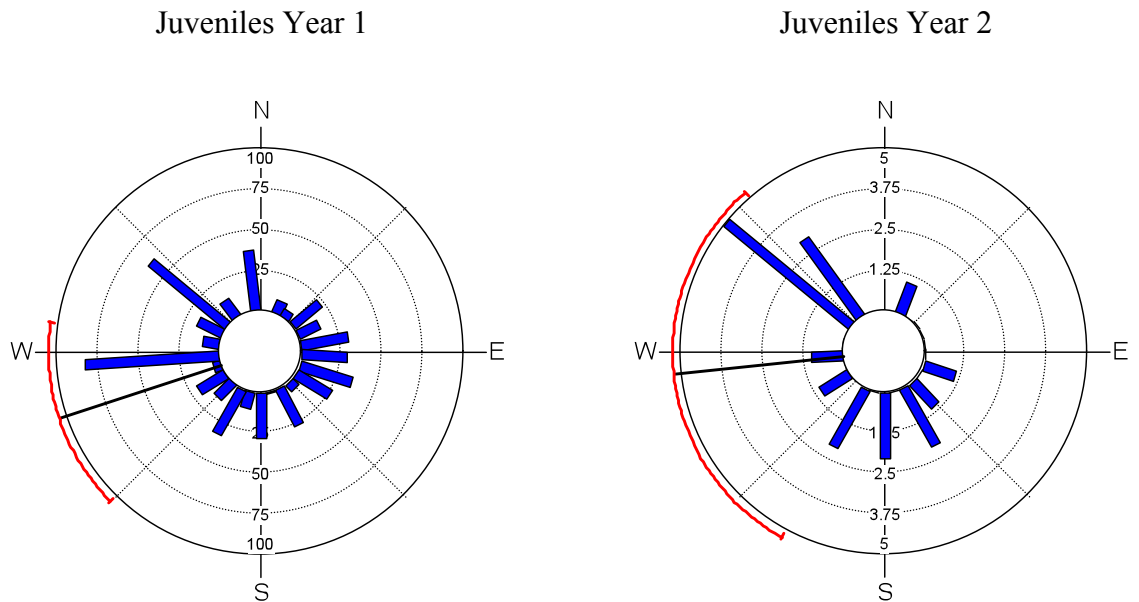


Figure 14. Orientation of emigrating juvenile *A. maculatum* at site 26007.

The black line indicates the mean direction of movement and 95% confidence intervals.

Red confidence intervals may be unreliable due to the lack of concentration.

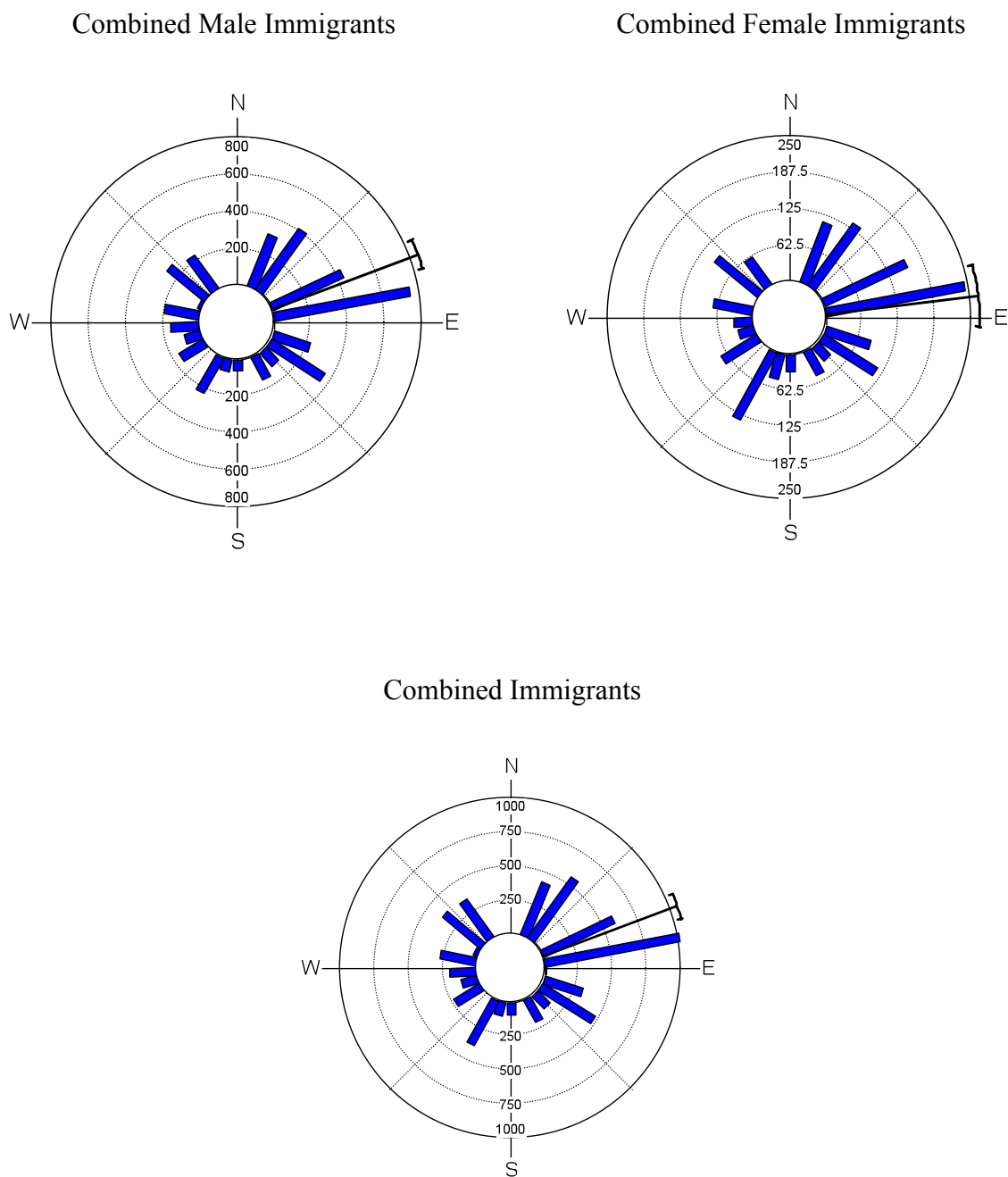


Figure 15. Male and female *A. maculatum* immigration orientation at site 26007.

The black line indicates the mean direction of movement and 95% confidence intervals.

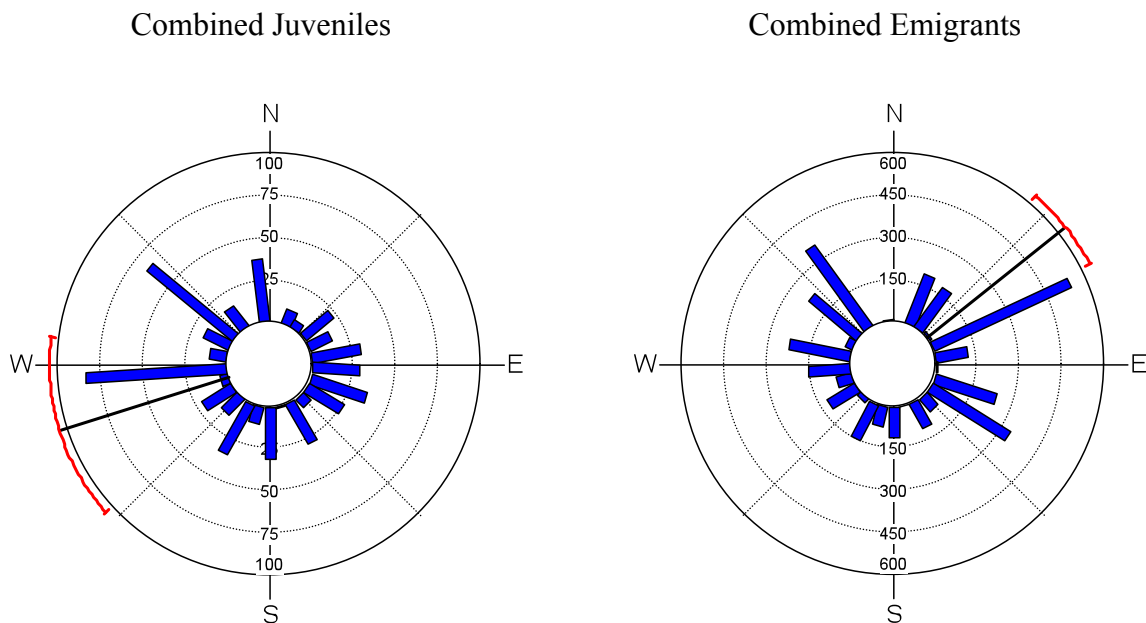


Figure 16. Orientation of combined juvenile and emigrating *A. maculatum* at site 26007.

The black line indicates the mean direction of movement and 95% confidence intervals.

Red confidence intervals may be unreliable to the lack of concentration.

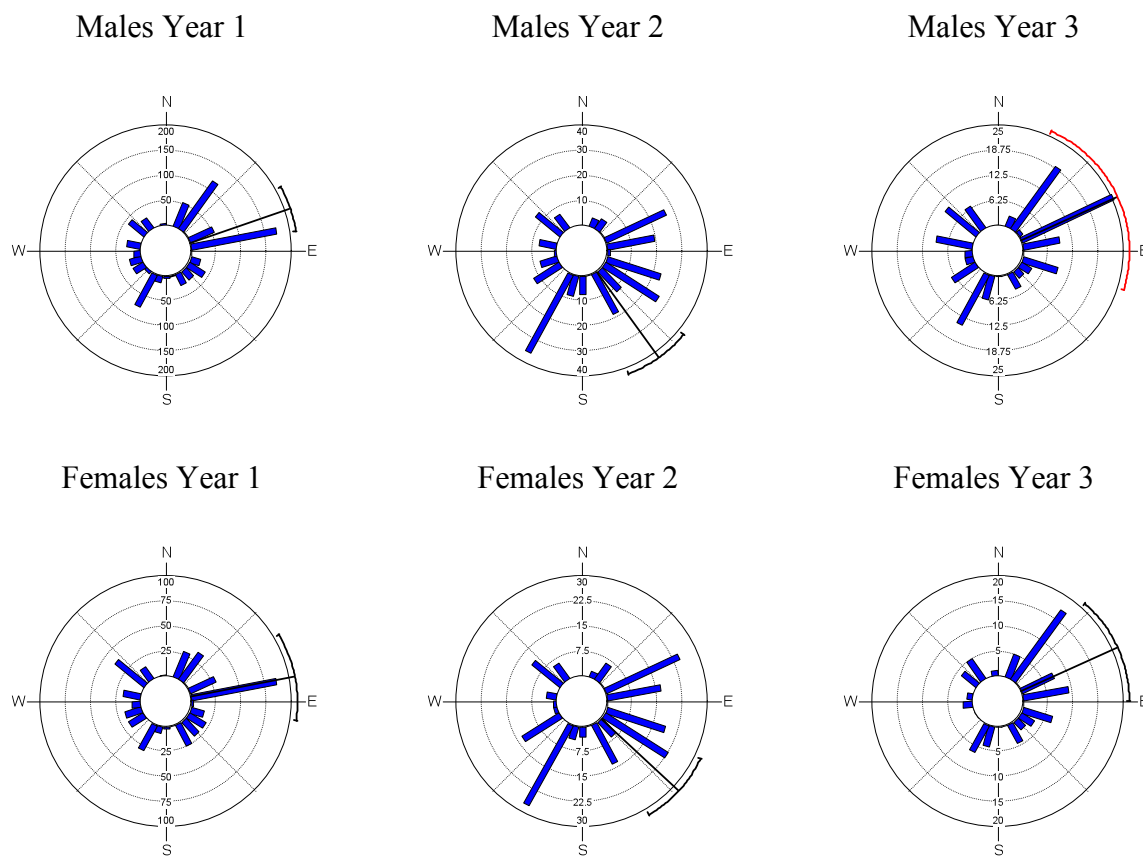


Figure 17. Orientation of immigrating adult male and female *A. talpoideum* at site 26007.

The black line indicates the mean direction of movement and 95% confidence intervals.

Confidence intervals that are red may be unreliable due to the lack of concentration.

348.277, $p < 0.01$, Year 2 $U = 342.902$, $p < 0.01$, Year 3 $U = 331.542$, $p < 0.01$, Fig. 18). Distributions of juvenile emigrations were not uniformly distributed across the site during any year (Year 1 ($U = 341.520$, $p < 0.01$), Year 2 ($U = 357.486$, $p < 0.01$), Fig. 19). All combined immigrations lacked uniformity (Combined Male $U = 351.885$, $p < 0.01$, Combined Female $U = 348.387$, $p < 0.01$, Combined Immigrants $U = 355.087$, $p < 0.01$, Fig. 20). Distributions for combined emigrations of juveniles and adults were not uniform (Combined Juveniles $U = 349.339$, $p < 0.01$, Combined Emigrants $U = 354.029$, $p < 0.01$, Fig. 21).

26031

Ambystoma opacum

Male and female *A. opacum* immigrations lacked uniformity at site 26031 across all years (Male Year 2 $U = 282.000$, $p < 0.01$, Male Year 3 $U = 240.000$, $p < 0.01$, Female Year 2 $U = 341.511$, Female Year 3 $U = 302.400$, $p < 0.01$, Fig. 22). Juvenile and adult emigrations also differed from parity at site 26031 (Juvenile Year 1 $U = 356.398$, $p < 0.01$, Juvenile Year 2 $U = 352.733$, $p < 0.01$, Adult Emigrants Year 2 $U = 321.322$, $p < 0.01$, Adult Emigrants Year 3 $U = 300.00$, $p < 0.01$, Fig. 23). Analyses of combined male, female and all immigrant movements lacked uniformity (Combined Males $U = 304.078$, $p < 0.01$, Combined Females $U = 344.814$, $p < 0.01$, Combined Immigrants $U = 348.539$, $p < 0.01$, Fig. 24). Combined Juvenile emigrations were not uniformly distributed across the site ($U = 357.479$, $p < 0.01$) and combined emigrant movements also lacked uniformity ($U = 340.661$, $p < 0.01$, Fig. 25).

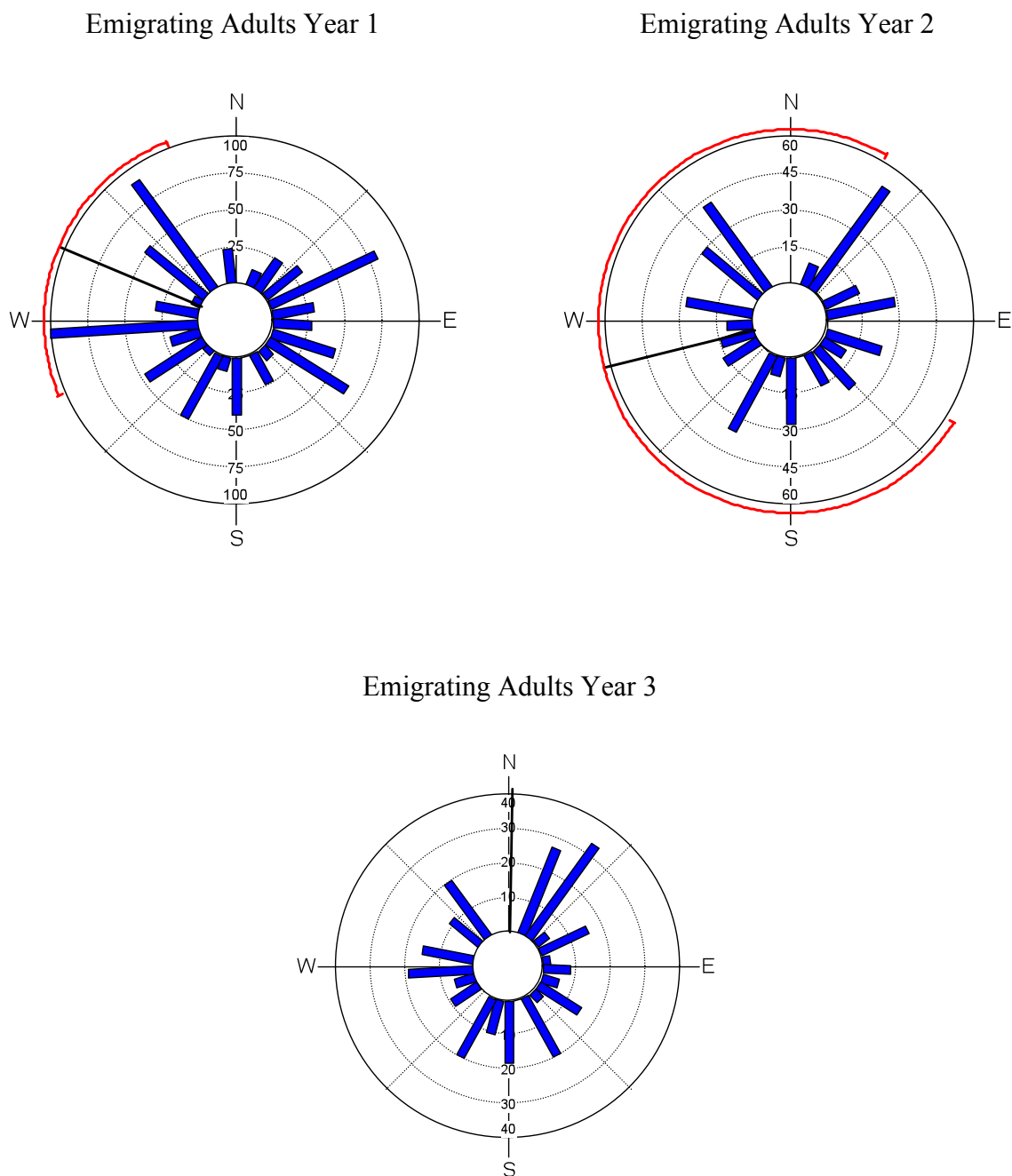


Figure 18. Orientation pattern of emigrating adult *A. talpoideum* at site 26007. The black line indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable due to the lack of concentration.

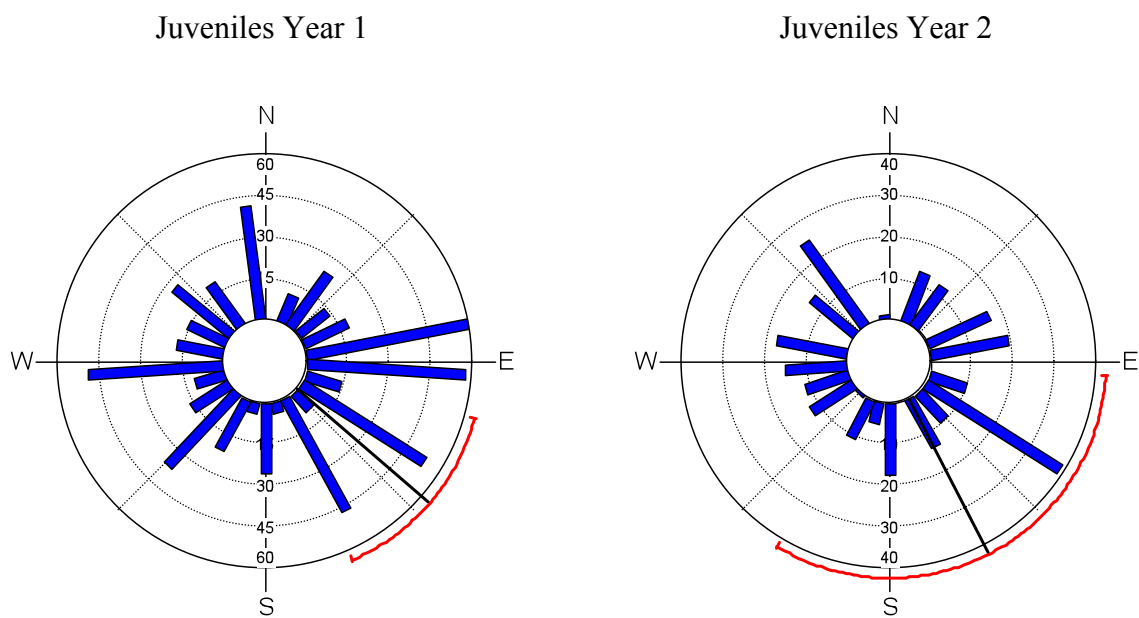


Figure 19. Orientation of emigrating juvenile *A. talpoideum* from site 26007. The black line indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable due to the lack of concentration.

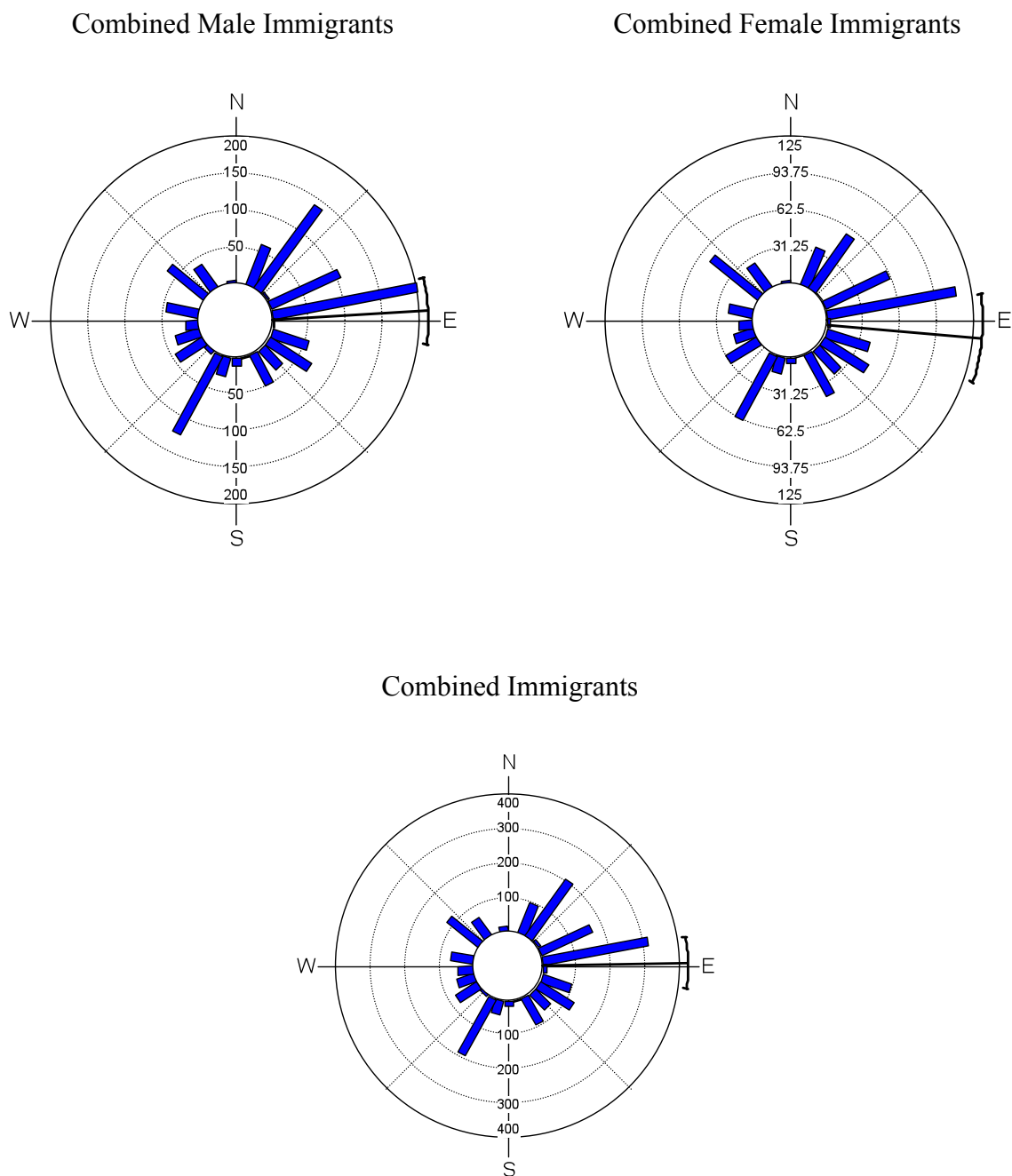


Figure 20. Orientation for combined male and female *A. talpoideum* immigrating at site 26007. The black line indicates the mean direction of movement, and 95% confidence intervals.

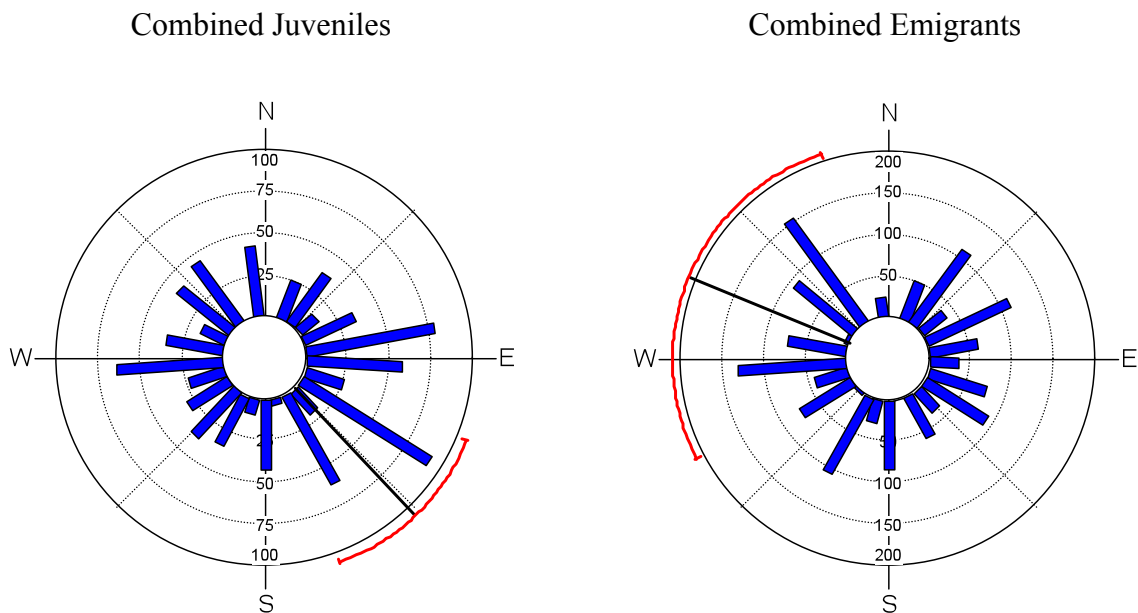


Figure 21. Orientation of combined juvenile and combined emigrating *A. talpoideum* from site 26007. The black line indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable to the lack of concentration.

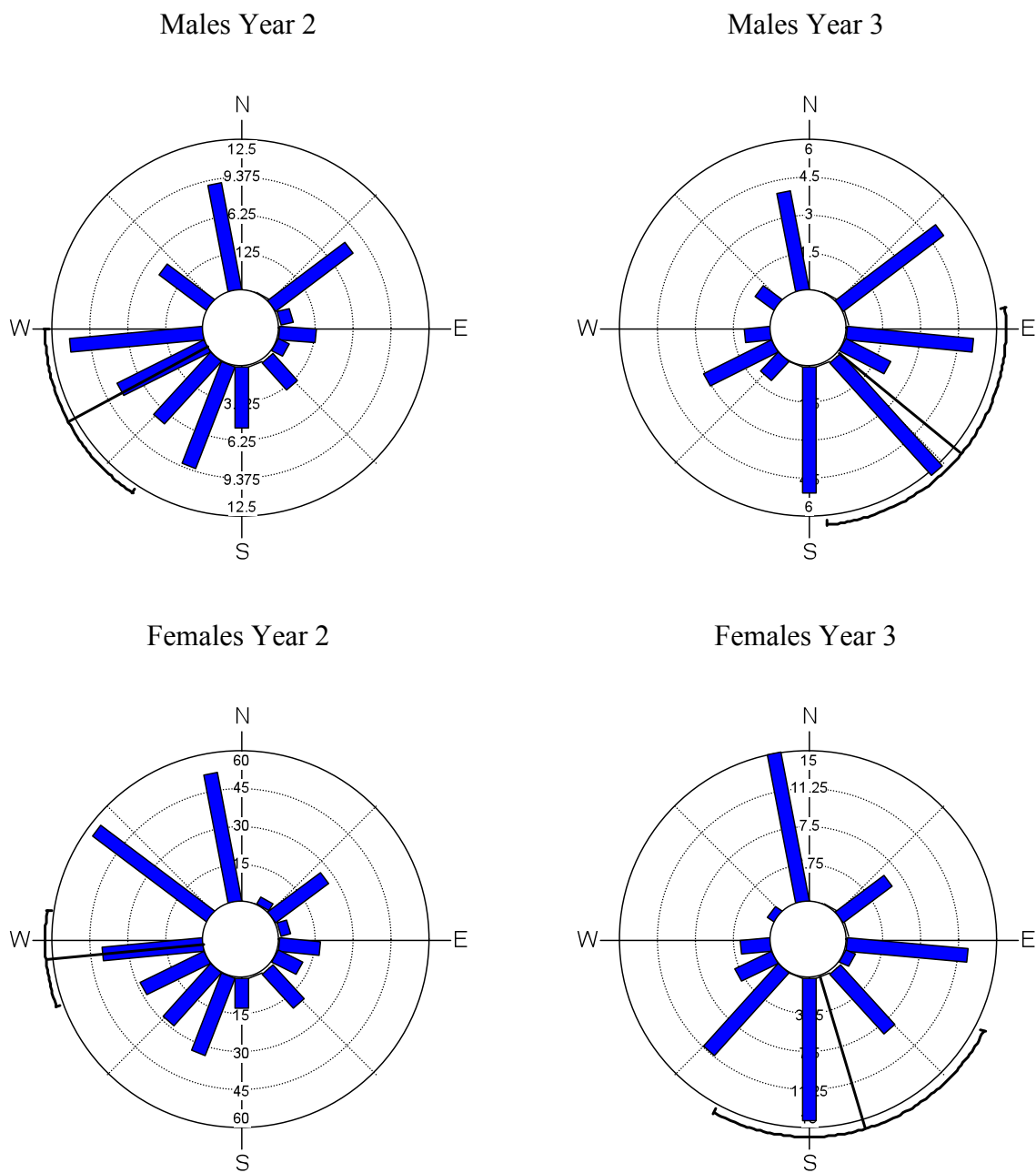


Figure 22. Orientation of adult female and male *A. opacum* at site 26031 during Year 2 and Year 3. The solid black line indicates the mean direction of movement, and 95% confidence intervals.

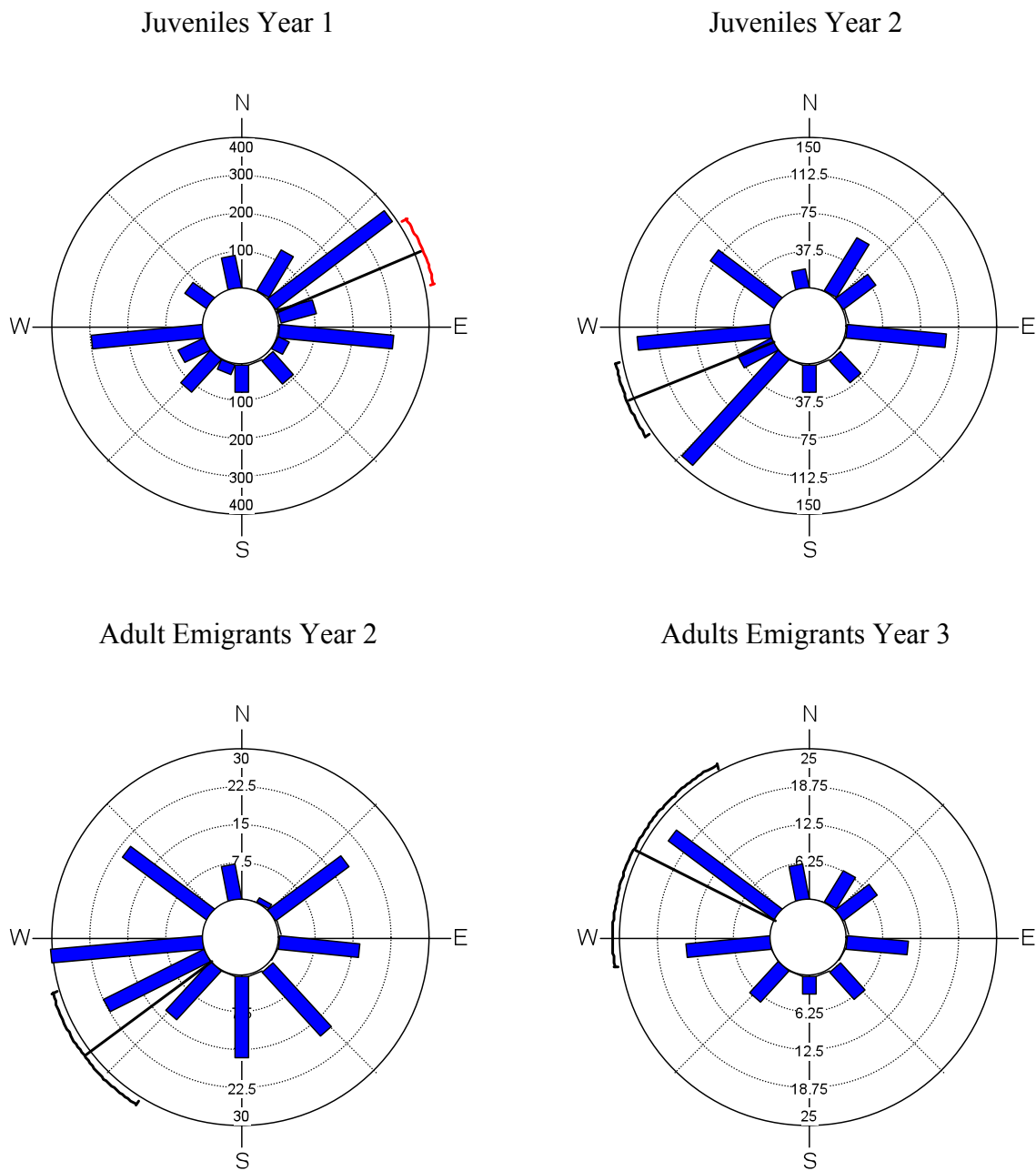


Figure 23. Migratory orientation of juvenile and emigrating adult *A. opacum* at site 26031. The black line indicates the mean direction of movement, and 95% confidence intervals. Red confidence intervals may be unreliable due to the lack of concentration.

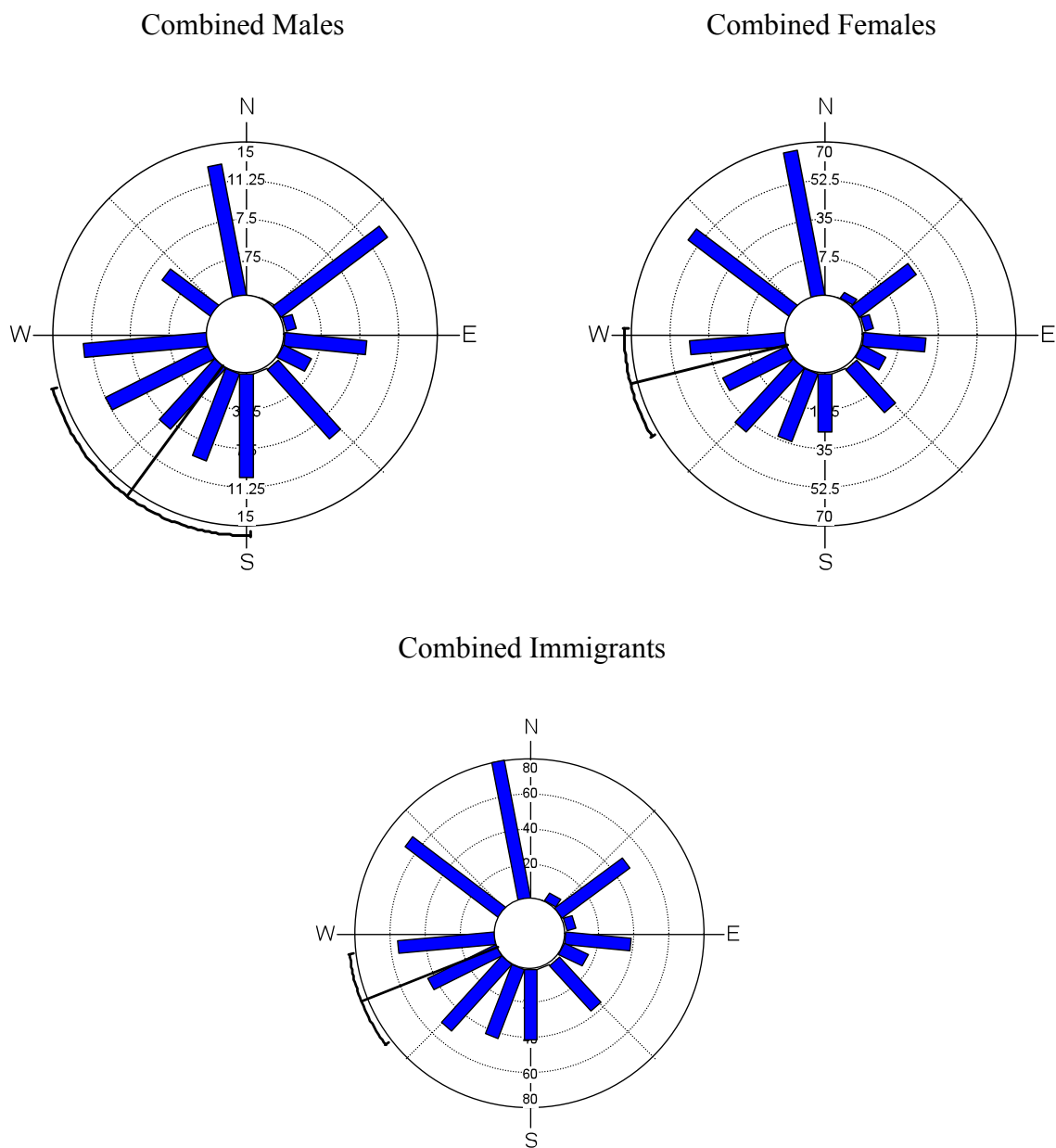


Figure 24. Orientation of combined male, female, and adult *A. opacum* immigrating at site 26031. The black line indicates the mean direction of movement, and 95% confidence intervals.

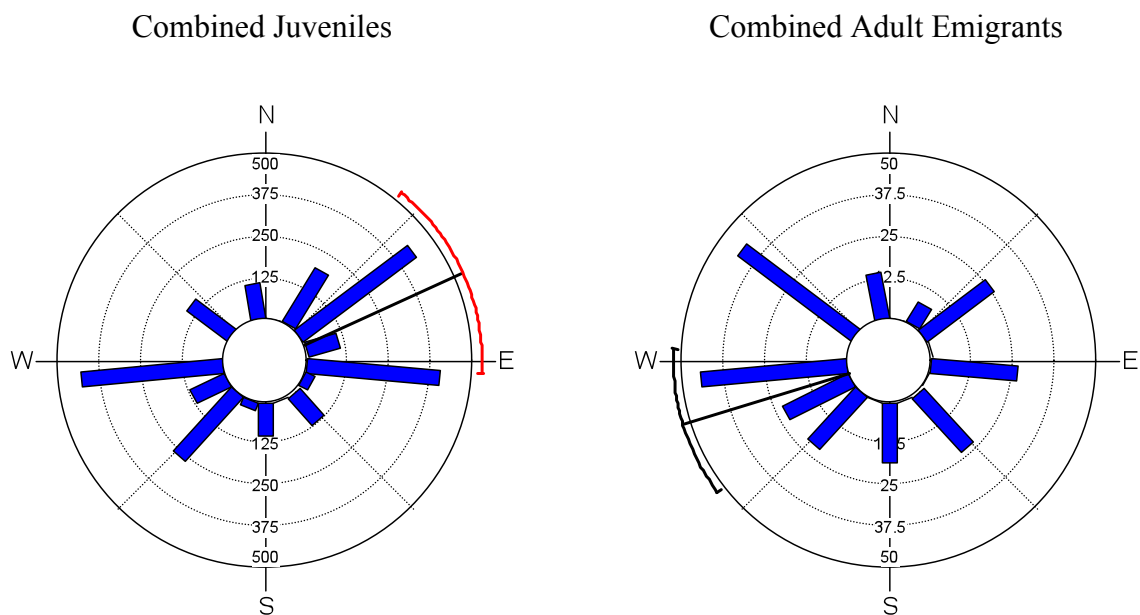


Figure 25. Orientation of combined juvenile and combined emigrating *A. opacum* at site 26007. The black line indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable to the lack of concentration.

Ambystoma maculatum

Male and female *A. maculatum* migrations differed from parity across all years at site 26031 (Male Year 1 $U = 344.051$, $p < 0.01$, Male Year 2 $U = 312.727$, $p < 0.01$, Male Year 3 $U = 312.245$, $p < 0.01$, Female Year 1 $U = 337.021$, $p < 0.01$, Female Year 2 $U = 312.857$, Female Year 3 $U = 312.727$, $p < 0.01$, Fig. 26). Juvenile migrations were not uniform during either year (Year 1 $U = 307.059$, $p < 0.01$, Year 2 $U = 218.390$, $p < 0.01$, Fig. 27). Adult emigrations from the site were not uniform during any year (Adult Emigrants Year 1 $U = 341.714$, $p < 0.01$, Adult Emigrants Year 2 $U = 311.786$, $p < 0.01$, Adult Emigrants Year 3 $U = 320.315$, $p < 0.01$, Fig. 27). Combined male and female immigrants and all combined immigrant movements were not uniform at the site (Combined Male $U = 348.837$, $p < 0.01$, Combined Female $U = 346.22$, $p < 0.01$, Combined Immigrants $U = 353.93$, $p < 0.01$, Fig. 28). Both combined juveniles and combined emigrant movements lacked uniform distributions (Combined Juveniles $U = 315.971$, $p < 0.01$, Combined Emigrants $U = 352.369$, $p < 0.01$, Fig. 29).

Ambystoma talpoideum

Ambystoma talpoideum male and female immigrations to site 26031 were not uniform during any year (Male Year 1 $U = 327.821$, $p < 0.01$, Male Year 2 $U = 274.286$, $p < 0.01$, Male Year 3 $U = 265.263$, $p < 0.01$, Female Year 1 $U = 340.418$, $p < 0.01$, Female Year 2 $U = 291.064$, $p < 0.01$, Female Year 3 $U = 293.023$, $p < 0.01$, Fig. 30). Juvenile migrations were not uniform during Year 1 ($U = 343.784$, $p < 0.01$), but were during Year 2 ($U = 188.180$, $p < 0.05$, Fig. 31). Year 2 results are unreliable due to the low sample size. Distributions of annual adult emigrations from the site were not uniform (Year 1 $U = 346.027$, $p < 0.01$, Year 2 $U = 326.512$, $p < 0.01$, Year 3

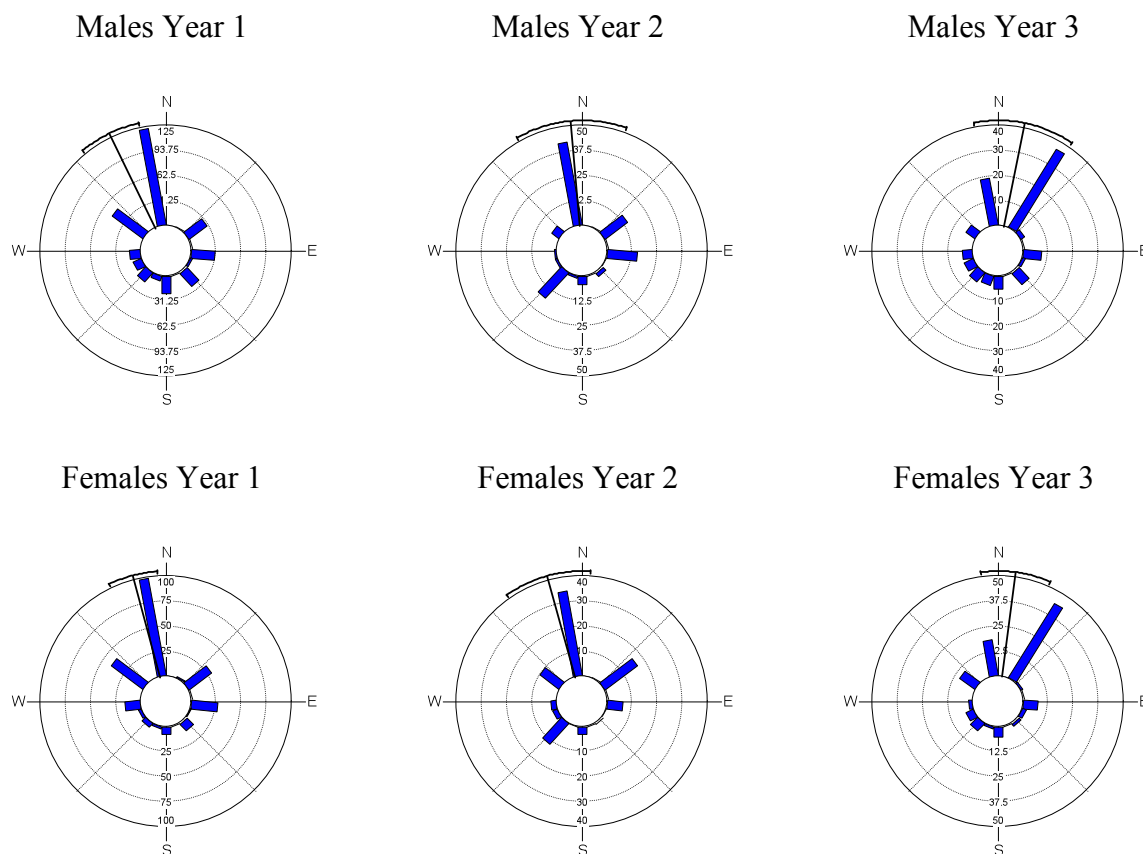


Figure 26. Orientation of immigrating adult male and female *A. maculatum* at site 26031.

The black line indicates the mean direction of movement and 95% confidence intervals.

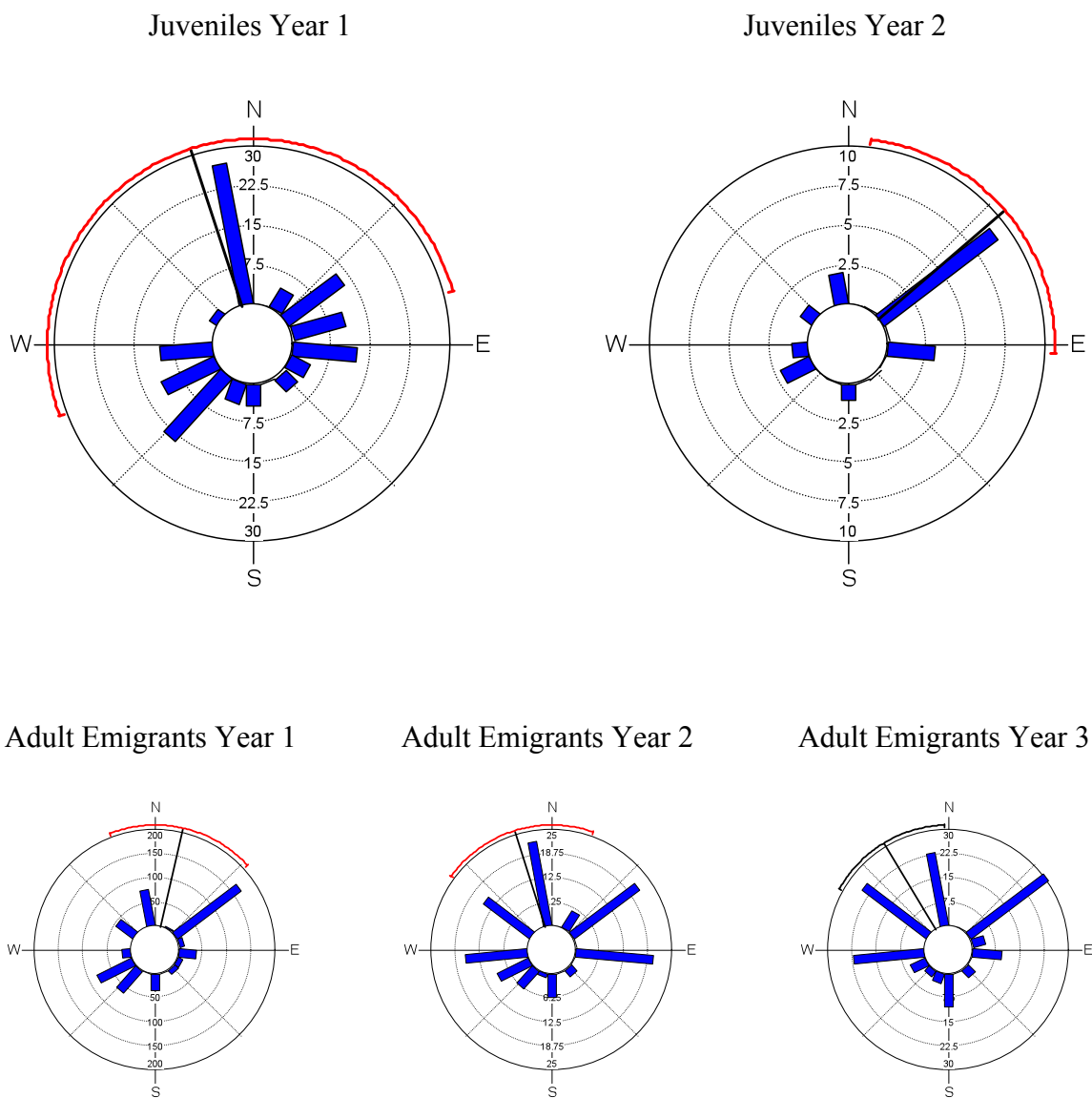


Figure 27. Orientation of emigrating juvenile and adult *A. maculatum* at site 26031. The black line indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable due to the lack of concentration or a small sample size.

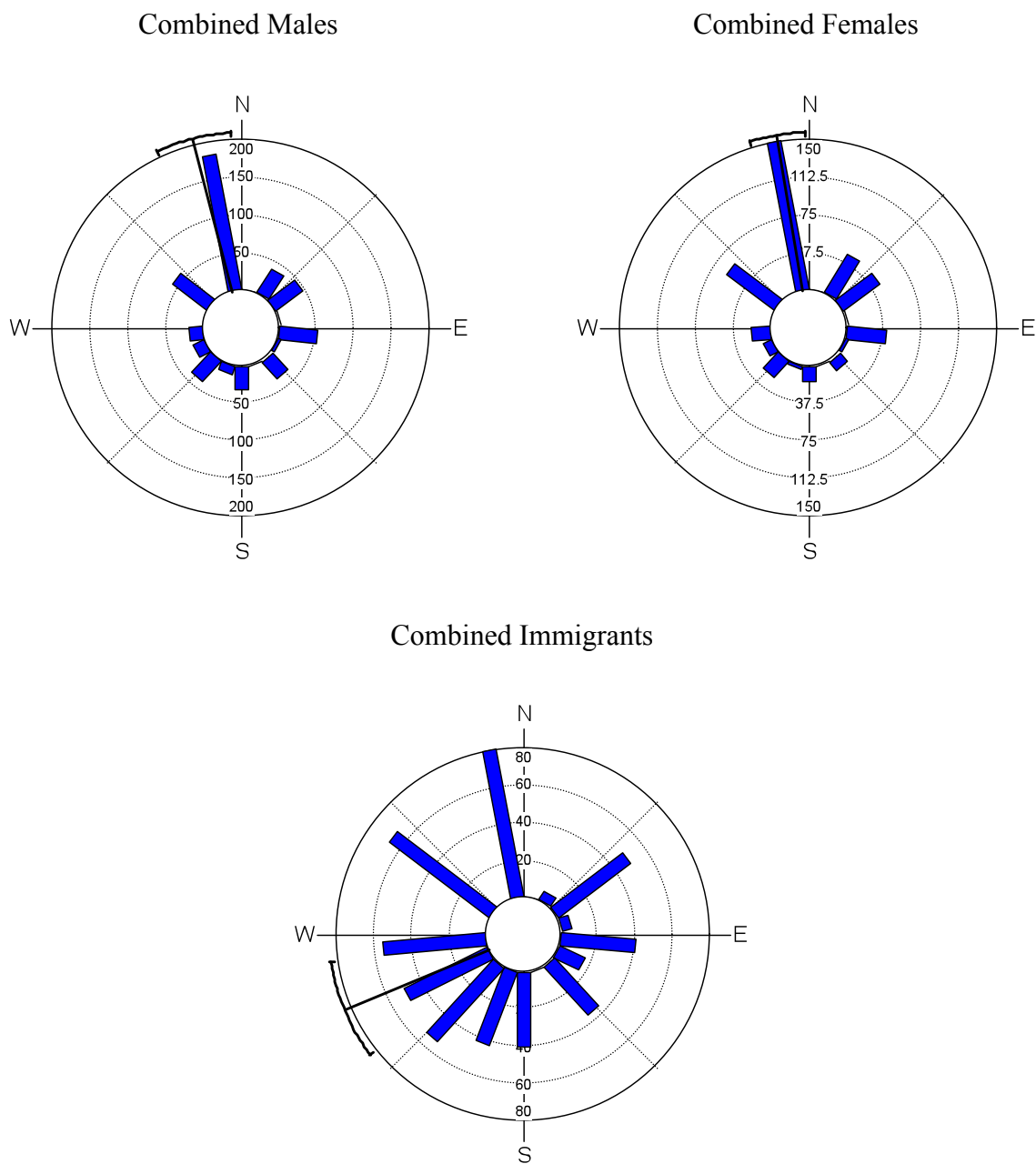


Figure 28. Immigration orientation for combined male and female and combined adult *A. maculatum* at site 26031. The black line indicates the mean direction of movement, and 95% confidence intervals.

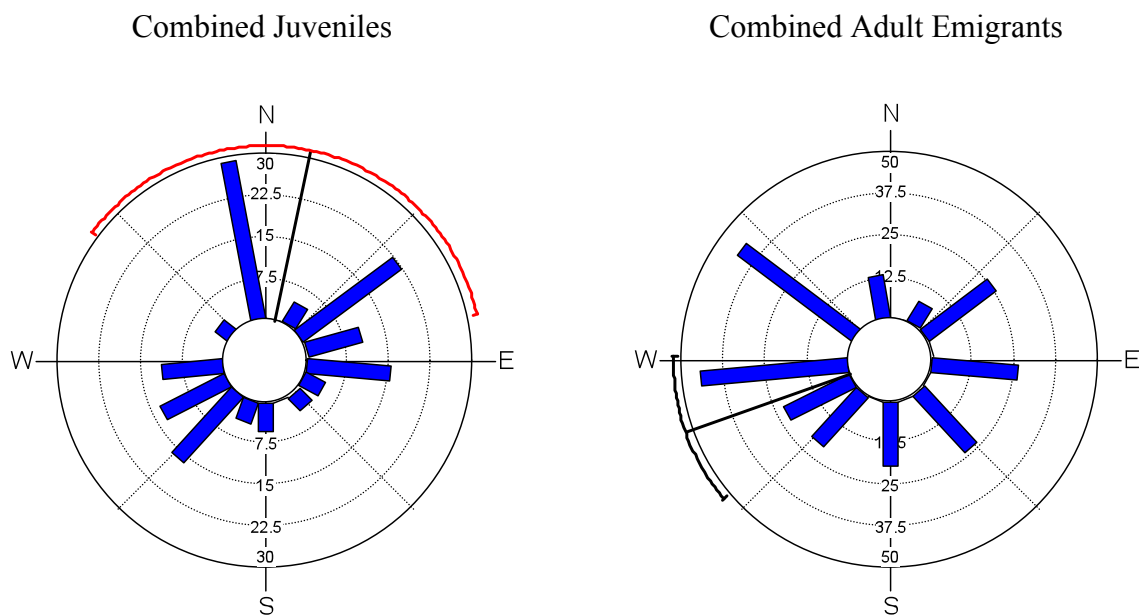


Figure 29. Orientation of emigrating juvenile and adult *A. maculatum* at site 26031. The black indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable due to a lack of concentration.

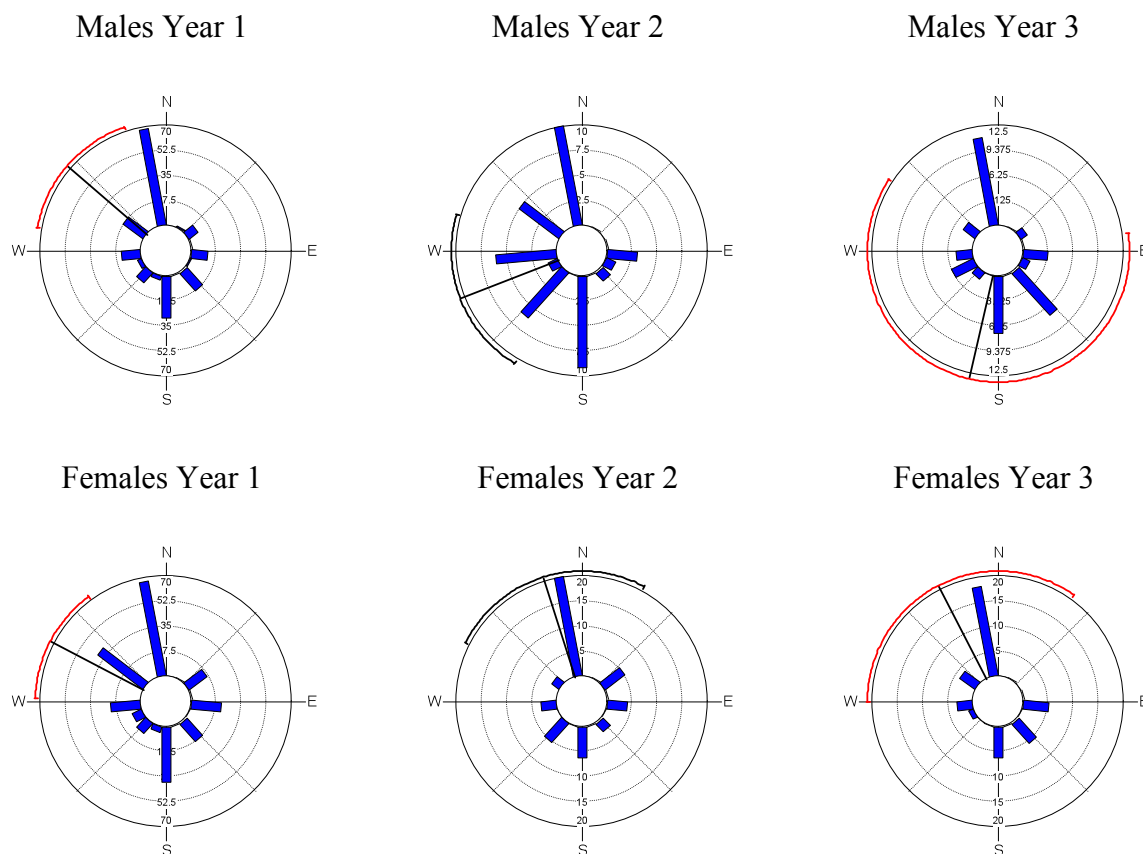


Figure 30. Immigration orientation of adult male and female *A. talpoideum* at site 26031.

The black line indicates the mean direction of movement and 95% confidence intervals.

Red confidence intervals in red may be unreliable due to a lack of concentration.

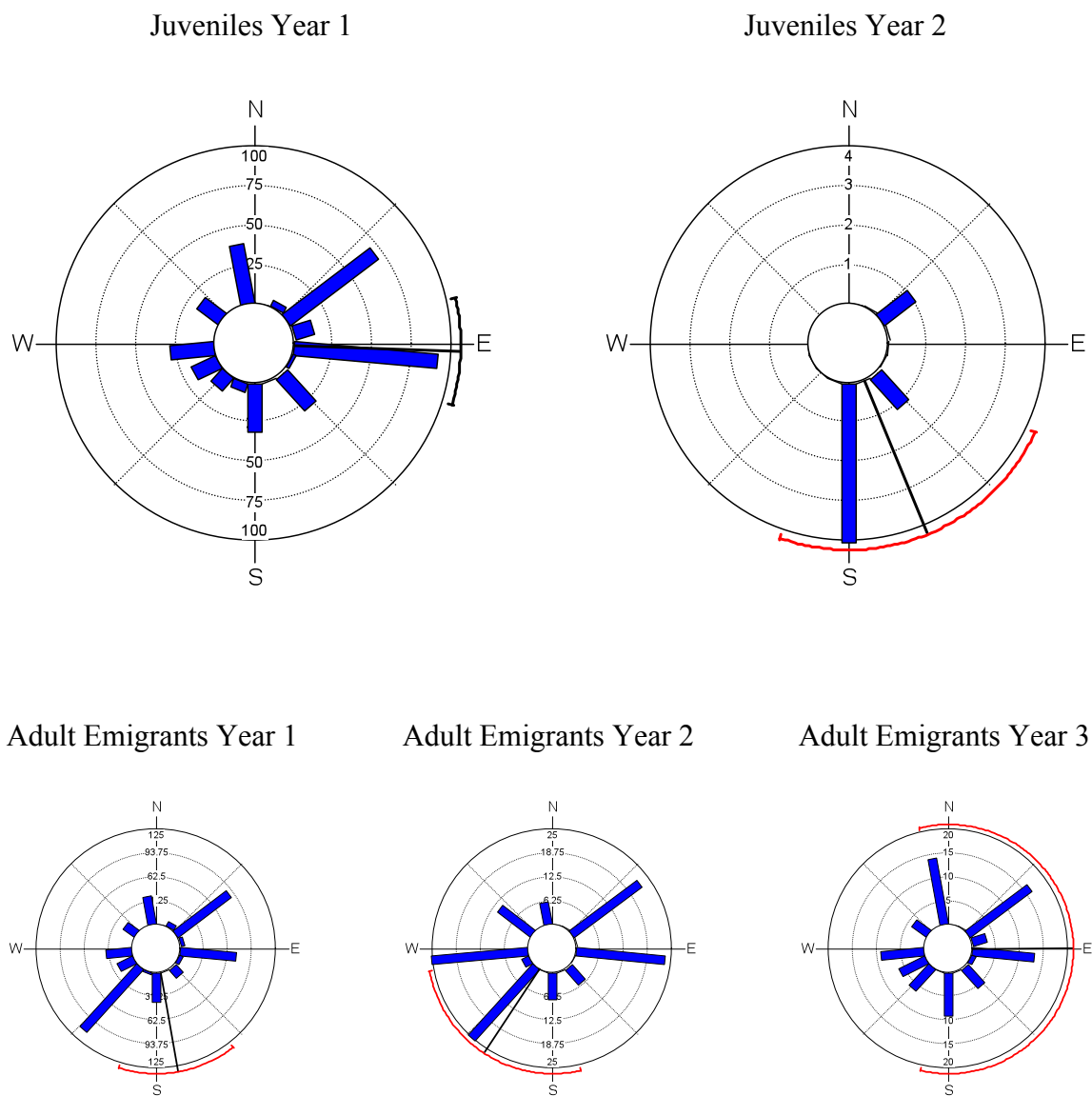


Figure 31. Orientation of juvenile and emigrating adult *A. talpoideum* at site 26031. The black line indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable due to a lack of concentration or a small sample size.

$U = 304.941, p < 0.01$, Fig. 31). Analyses of combined male and female immigrants and all combined immigrants were not uniformly distributed across the site (Combined Male $U = 337.814, p < 0.01$, Combined Female $U = 345.732, p < 0.01$, Combined Immigrants $U = 350.415, p < 0.01$, Fig. 32). Combined juveniles ($U = 343.982, p < 0.01$) and combined adult emigrant movements lacked uniformity ($U = 350.768, p < 0.01$) (Fig. 33).

Discussion

Distributions of migrations were not uniform among species of ambystomatids, sexes of the species, age class, and year. The lack of uniformity observed at each site is not unusual. Migratory behaviors such as these are common (Shoop 1968; Douglas and Monroe 1981; Jenkins *et al.* 2006) and have been observed elsewhere in Tennessee (Wyckoff 2006). Prior to the study, it was unknown how habitat modifications surrounding site 26031 were affecting salamander dispersals and orientation to and from the site. Given the magnitude of the modifications to the habitat at site 26031, amphibians had to migrate through the clearcut to reach the breeding site. Todd *et al.* (2009) determined that clearcuts greater than 4 ha could act as movement barriers to salamanders, and Rittenhouse and Semlitsch (2006) observed *A. maculatum* shift orientation from grassland habitat to forested areas indicating a preference for forested habitats. It was hypothesized that few salamanders bred at site 26031 because of the habitat alterations and the known avoidance of disturbed habitats by salamanders (Semlitsch *et al.* 2009). Approximately 11.2 ha surrounding site 26031 were disturbed

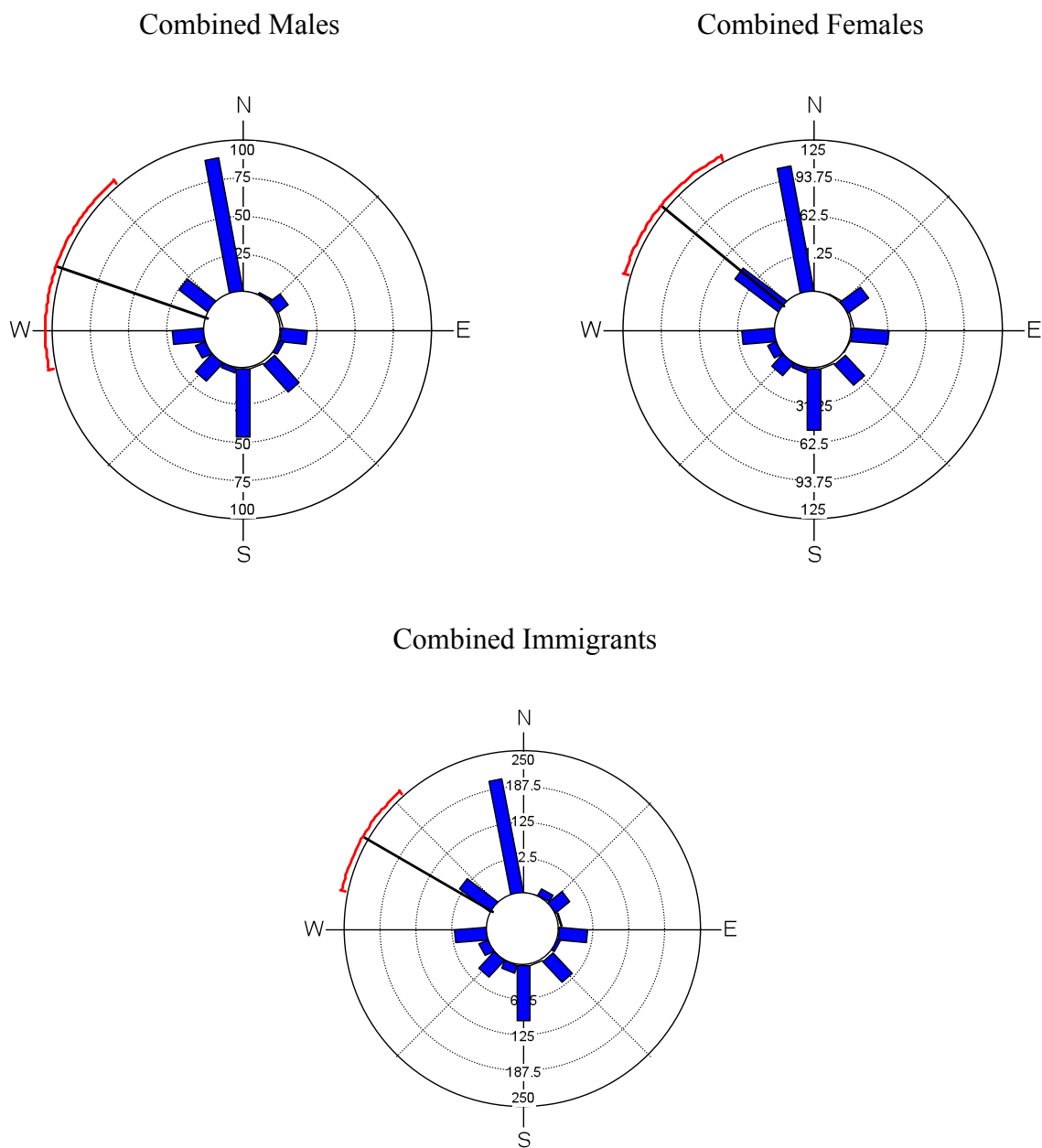


Figure 32. Immigration orientation of male, female, and combined adult *A. talpoideum* at site 26031. The black line indicates the mean direction of movement, and 95% confidence intervals. Red confidence intervals may be unreliable due to a lack of concentration.

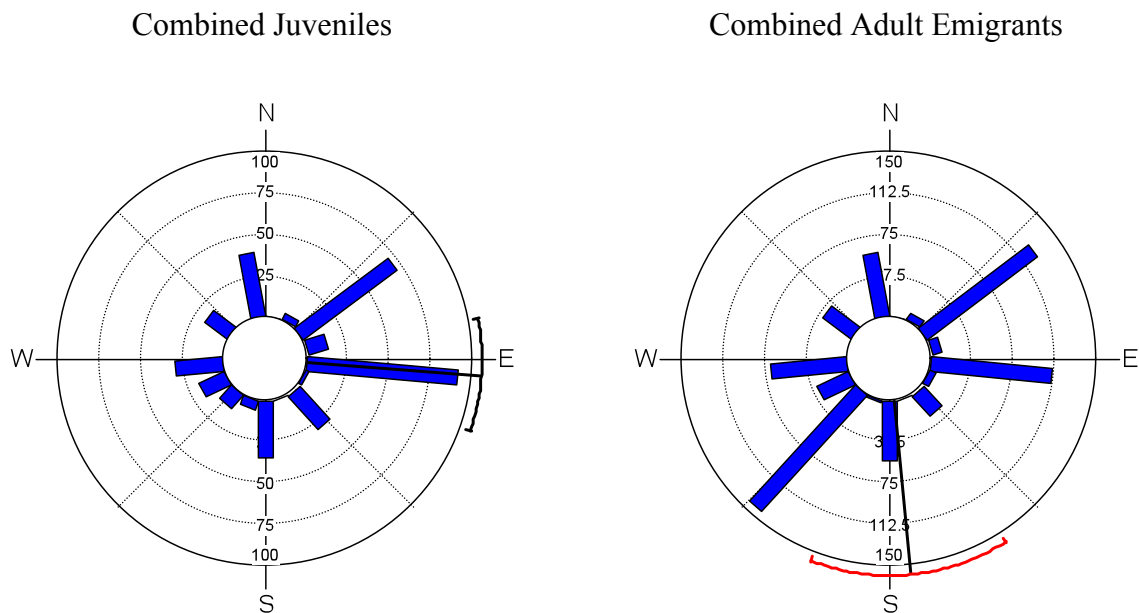


Figure 33. Orientation of combined juvenile and combined emigrant *A. talpoideum* at site 26031. The black indicates the mean direction of movement and 95% confidence intervals. Red confidence intervals may be unreliable due to a lack of concentration.

prior to and during the study, and results indicate these alterations have not disrupted adult salamander migrations at the site; however, it is not known how the migratory behavior of the salamander population may have been impacted by the disturbance of the terrestrial habitat prior to this study (telemetry data presented below).

Non-uniform movements by migrating salamanders to breeding sites are often associated with habitat quality. Todd *et al.* (2009) showed forested habitats were chosen most often by *A. talpoideum* during emigration compared to clearcut treatments, but uniform distributions were observed during immigrations, presumably because of similar environmental conditions across treatments. Raymond and Hardy (1991) observed immigration of adult *A. talpoideum* decreased annually along portions of a breeding site. Decreases were attributed to a clearcut and subsequent creation of monoculture pine plantation. Regosin *et al.* (2005), working with two species of *Ambystoma*, found non-uniform migrations associated with the amount of forested habitat surrounding the breeding site. Although the appropriate habitat might exist around disturbed breeding habitats, studies have found uniform salamander migrations or are unable to explain non-uniform movements. Beneski *et al.* (1986) found *A. macrodactylum* migrated equally through all five available habitats located around their study site in northern Idaho. Johnson (2003) detected non-uniform movements in a population of *Notophthalmus perstriatus*, but was unable to determine the existence of habitat influencing migrations. Both uniform and non-uniform migrations were observed in the three species of ambystomatid studied at Bear Hollow Mountain WMA, and migration patterns varied between species, sex, and age. Differences in migratory patterns observed among the species may have been due to habitat features surrounding the sites.

Mean vectors from Rao's Spacing Tests varied among all three species of ambystomatids and years. *A. opacum* primarily entered and exited site 26031 using bearings from 180° – 270° and western vectors at site 26007. Entering from these bearings minimizes the distance of migration at site 26031, as the western portion of the breeding site is closest to the forest edge. Shorter migration distances minimize the time migrating salamanders are exposed to the harsh conditions of the clearcut. Also, wet weather conveyances are located along the western portions of each site, likely maintaining moisture regimes necessary for successful migrations. These moisture regimes would be especially important to *A. opacum*, which immigrated to the site as early as August along vectors similar to that of the wet weather conveyances. Migrations during this time of the year come at high costs associated with increased temperatures and water losses salamanders would endure. Presumably, the wet weather conveyances maintain moisture regimes in the late summer and early fall and enable migrations to occur.

At site 26007, *A. maculatum* and *A. talpoideum* immigrated from the north and east and emigrated north and east. Because of the lack of observed habitat differences at this site, it is difficult to ascertain the exact reason for these migratory concentrations. The location of a second wetland, known to support breeding populations of ambystomatids, may possibly explain why these two species migrate in this fashion. The majority of the population for each of these species may be located in habitats located north and east of these two wetlands. Also, there are a number of depressions located north and east of these wetlands that do not support breeding, but may maintain moisture regimes sufficient enough to support individuals outside the breeding season.

Because of sensitivities associated with moisture regimes, ambystomatids may readjust orientation within upland habitats. As a result, vectors identified in the study may not accurately reflect habitat choices outside of the breeding season. Jenkins *et al.* (2006) found that salamanders often changed orientation from the breeding site while traveling in upland habitats, possibly in response to preferable microenvironments. These microenvironments may be more preferable for migration if these areas have cooler temperatures and higher moisture regimes. High concentrations of captures at breeding sites may not indicate travel corridors for amphibians at breeding sites, and careful consideration should be given when using this type of data for determining the appropriate protection measures.

Small mammal burrows are an important habitat for ambystomatid salamanders, providing both thermal and moisture refuge (Rothermel and Luhring 2005). Faccio (2003) found that *A. maculatum* used small mammal burrows almost exclusively as terrestrial refuges, highlighting the importance of this habitat characteristic. Removal of coarse woody debris and pine litter has little effect on the activity levels of *A. talpoideum* (Moseley *et al.* 2004), and the lack of these features within a landscape may be offset by the presence and density of small mammal burrows. Steen *et al.* (2006) suggested that Oldfield Mice (*Peromyscus polionotus*) were important for creating subterranean habitat for *A. tigrinum*. Furthermore, Regosin *et al.* (2003) found *A. maculatum* was less likely to use areas void of small mammal burrows, and that the density of small mammal burrows may impact salamander density within a habitat. Possibly, the abundance of salamanders observed at each breeding site at Bear Hollow Mountain WMA are indicative of small mammal burrow density within the area, the density and location of

burrows across each site is unknown. Future studies should seek to determine if a correlation exists at each site between small mammal burrows and salamander abundance.

There was distinct segregation around both sites by ambystomatids during migration. It is unknown if salamanders were using specific portions of habitat surrounding each site having characteristics advantageous for successful migration, and no data were collected to determine if such characteristics were present. Temperature and moisture cues may aid the ability of salamanders to select non-breeding habitat (Rittenhouse *et al.* 2004); however, migration occurs during cloudy and rainy nights when moisture regimes are similar among preferred and non-preferred habitats. Also, migrations of most ambystomatids occur during the late winter and early spring when air and soil temperature are similar between forested and non-forested habitats (Chen *et al.* 1997). *Ambystoma maculatum* have the ability to select preferred habitat when given multiple choices (Rittenhouse *et al.* 2004). The exact cues salamanders use to select habitat is unknown, but soil composition cues presumably aid selection. It is not known what, if any, differences exist with soil composition at either site studied at Bear Hollow Mountain WMA, or if unknown differences contribute to distributions of the salamanders around the sites.

Although habitat alterations, such as those taking place at site 26031, have been shown to negatively impact and influence salamander migrations, ongoing management apparently is not negatively impacting migrations at site 26031 as salamanders travel successfully through the clearcut. Because uniformity varied between species annually at each site, habitat alterations appear not to be influencing how salamanders orientate to site 26031; however, these results do not necessarily indicate this type of habitat

management surrounding amphibian breeding sites goes without negative impacts.

Affects of habitat alterations on the predation rates of salamanders during migrations at site 26031 are unknown. Presumably, predation rates increase because salamanders are migrating through an open habitat with considerably higher amounts of bare soil increasing exposure to potential predators. Successful emigrations of juveniles from the breeding site to the forest were not assessed and should be considered in future studies. This research only indicated when juveniles began emigration and did not determine potential impacts of current habitat alterations. Currently, this research only indicates habitat alterations of this type and size does not appear to impact the migration and orientation of salamanders to breeding sites on the Southern Cumberland Plateau.

Chapter 3: Telemetry

Spotted Salamanders (*A. maculatum*) have a large geographic range comprising much of the eastern United States and extending west of the Mississippi (Petranka 1998; Lannoo 2005). Despite this widespread geographic occurrence, *A. maculatum* has, and continues to suffer, range reductions due to habitat alterations (Lannoo 2005).

Conservation strategies for this species must take into consideration the species life cycle (Semlitsch 1998). *Ambystoma maculatum* spends much of the year in terrestrial forested habitat and migrates to temporary wetlands where breeding and development of larvae occurs. Strategies employed to protect the species must adequately protect both terrestrial and aquatic habitats.

A literature review on terrestrial habitat use by six species of *Ambystoma* conducted by Semlitsch (1998) indicated federal delineated wetland boundaries and state recommended buffer zones were inadequate in protecting most populations of *Ambystoma* species. Federal and state protection measures were found to be inadequate because most adults and newly metamorphosed salamanders live in terrestrial habitat at distances beyond those recommended to be conserved as buffer zones. Consequently, Semlitsch (1998) proposed protection measures be extended beyond current recommendations to protect larger portions of terrestrial habitat surrounding breeding pools, which he described as “life zones” necessary for the maintenance of both juvenile and adult life stages.

Suitable terrestrial and breeding habitat can be found throughout the state of Tennessee. Approximately 5.6 million of the 10.5 million hectares of the state are forested and the amount of young forests (0 – 10 year age class) increased slightly during 2005 - 2009 (Oswalt *et al.* 2012). The exact acreage of wetlands occurring in Tennessee is not known, but the Tennessee Department of Environment and Conservation (Tennessee Department of Environment and Conservation 1998), through analysis of multiple data sets, estimated that the base of wetland capability in the state exceeds 809,000 hectares; however, this estimate includes hydric soils, which if vegetated, can be considered jurisdictional wetlands. It is believed numerous wetland acres have previously been converted to non-wetland uses and cannot be considered wetlands. Further analysis by TDEC (1998) indicated the amount of vegetated wetlands found in the state was approximately 258,665 hectares, but this estimate was incomplete as analysis was ongoing.

Although the amount of forested land on the landscape has increased in Tennessee and conservation strategies are being deployed to protect wetlands, these strategies are inadequate to protect the “life zones” recommended by Semlitsch for ambystomatid salamanders. Current best management practices (BMPs) dictate that a buffer twenty-five (25) feet in width must remain along the edge of the water and the buffer increases in width as the slope of the site increases. This buffer is referred to as the management zone. Other BMPs for the management zone include the location of roads away from the management zone, minimize the number of roads within the management zone, not operating equipment within the management zone, and removing the tops of trees from management zone to not impede water flow. These BMPs are aimed at protecting the

water quality of the breeding pool, but they do not protect the forested habitat surrounding wetlands critical for juvenile and adult survival.

Migration distances of *A. maculatum* were assessed at two research sites on Bear Hollow Mountain WMA. The goals of the research were to 1) assess if BMPs enacted prior to the State of Tennessee taking ownership were sufficient in protecting populations of *A. maculatum*, 2) determine if differences in the habitats surrounding the two wetlands impacted migration behavior, and 3) determine if current management practices implemented by TWRA were impacting the species.

Methods

Study Site

Adult female *A. maculatum* were captured using drift fence and pitfalls established during previous research conducted at sites 26007 and 26031 on Bear Hollow Mountain WMA. Pitfalls located on the interior of drift fences at both sites were opened on February 23, 2011 in an effort to capture adults exiting the breeding sites. To ensure animals selected for telemetry represented the entire site and were not skewed to a single portion of it, each site was broken into four quadrants, each quadrant containing no more than four pitfalls each (Fig. 34). No more than four animals were selected for telemetry from each quadrant to ensure all cardinal directions of migration were potentially represented. Pitfalls remained opened and checked every 1 - 3 days until all transmitters had been implanted or emigration ceased. Linear regression from previously collected data at each site was used to determine the appropriate size class of animals to select for telemetry. Upon capture, *A. maculatum* adult females meeting predetermined criteria for telemetry were collected and taken to a TWRA facility for implant surgery.

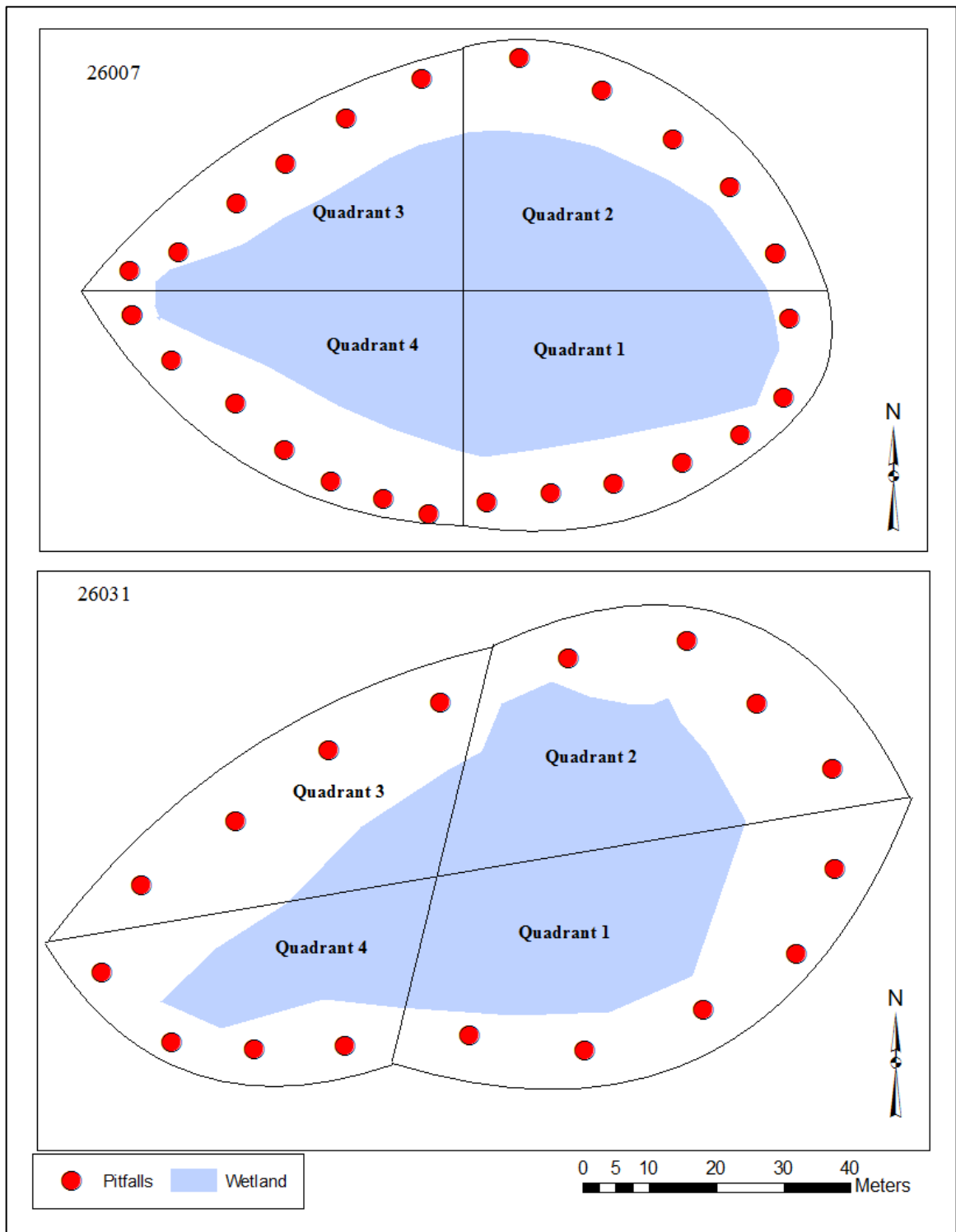


Figure 34. Locations of quadrants and all traps at sites 26007 and 26031 used in the selection of salamanders for radio-implantation.

Surgical Implantation Procedures

Ambystoma maculatum were anesthetized by being placed in a 0.02% benzocaine solution prepared by combining maximum strength Oragel ® (20% benzocaine) and distilled water. Individual *A. maculatum* were placed in a bath of the solution until movement ceased and animals lost the ability to right themselves. Surgical sites were sterilized with 3% hydrogen peroxide. A 2 - 4 mm longitudinal incision was made in the right ventrolateral abdominal wall between costal grooves approximately 10 – 12 cm anterior to the left hind leg. Salamanders were implanted with a model BD-2HX (Holohil Systems Ltd, Ontario, Canada) or a model PD-2 (Holohil Systems Ltd, Ontario, Canada) radio transmitter. Each BD-2HX transmitter weighed approximately 1.9 g and each PD-2 transmitter weighed 2.5 g. Each incision was closed with a single continuous suture (0 Vicryl™, Polyglactin 910, undyed, braided suture on a SH needle). Following surgical implantation, salamanders were placed in a recovery bath of distilled water and remained until movement was observed. Following recovery, salamanders were placed in plastic containers with damp, unbleached paper towels and held for a minimum of 24 hours. The Middle Tennessee State University Office of Compliance Institutional Animal Care and Use Committee approved all protocols (11-006).

Radiotracking Procedures

Implanted salamanders were released approximately 3 m from the edge of the pond in terrestrial habitat on days in which rain events were expected to minimize stress and facilitate movement. A R-1000 receiver (Communications Specialists, INC., Orange, California) with a three element Yagi antenna was used to locate the positions of salamanders during the day when migration movements had ceased. Locations of

salamanders were obtained every 1-3 days or following each rain event. A Garmin 76CSx GPS was used to record the location of each implanted salamander upon being located. Field tracking continued until batteries of transmitters failed or signal quality indicated the transmitters were beginning to fail. When batteries were beginning to fail, all efforts were made to unearth and collect the implanted salamanders.

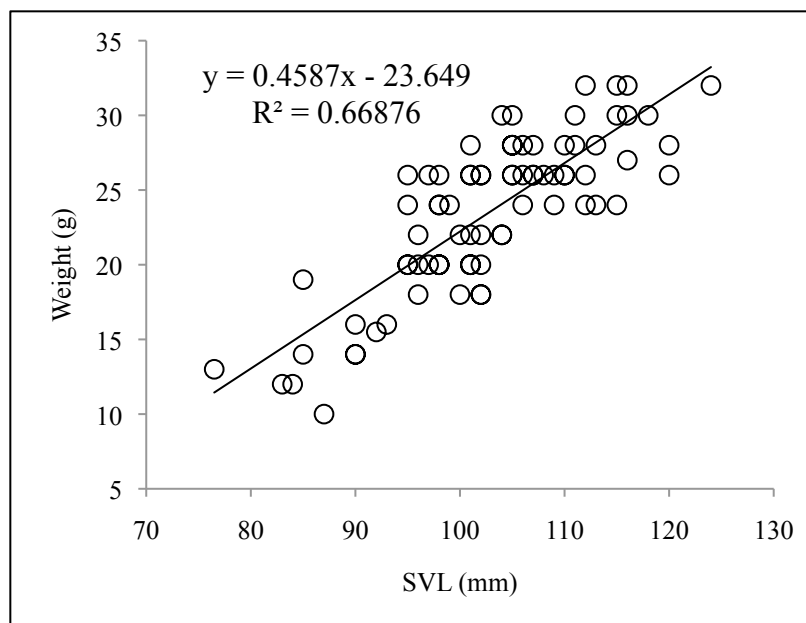
Results

Pitfalls remained opened through March 08, 2011 (10 trap nights). Although several rain events occurred during this trapping period, capture totals were low at both sites. Presumably, several breeding events took place prior to these trapping efforts as many egg masses were present at each site on February 23, 2011 when the pitfalls were opened.

Salamander Selection

Using data collected from 2007 – 2010, a linear regression was constructed for each research site (26007 and 26031) to aid in the determination of the suitability of captured salamanders for implantation. Only data for immigrating adult female *A. maculatum* were used to construct the linear regressions. It was determined the use of SVL at site 26007 ($SVL \geq 110.0\text{mm}$, $R^2 = 0.66876$) and TL (TL $\geq 195.0\text{mm}$, $R^2 = 0.1795$) at site 26031 would allow for the selection of salamanders suitable for implantation of radio transmitters (Fig. 35). These results indicated a salamander selected with a SVL ≥ 110.0 mm at 26007 would likely weigh 26.81 g and one selected

26007:



26031:

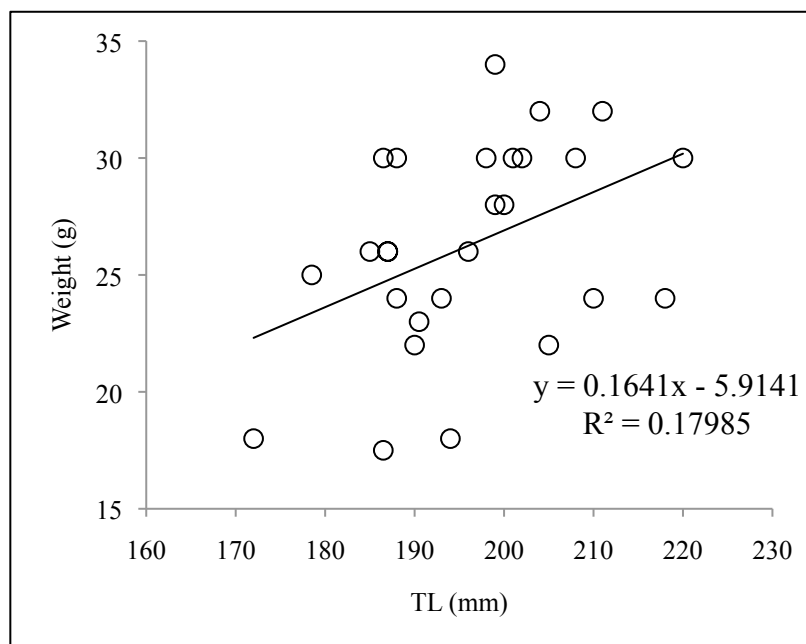


Figure 35. Linear regression models used to determine the appropriate size of female *A. maculatum* to select for implantation of transmitters.

with TL \geq 195.0 mm at 26031 would likely weigh 26.09 g or greater when immigrating to breeding sites. The use of salamanders of these sizes allows for the weight to radio transmitter ratio to be less than ten percent when taking into the account the pre-breeding migration weight. Samples sizes (26007 n = 80, 26031 n = 27) used in the linear regression were less than desirable. The low sample sizes, which are a result of a change in collection of weight and length data at both sites, explains why the correlation coefficient was significantly less at site 26031 when compared to site 26007.

Morphological data and radio transmitter data are summarized in Table 2. All *A. maculatum* selected at site 26007 fit in the criteria for implantation. Although the regression model predicted pre-breeding weights less than the post-breeding weights observed, the post-breeding weight to transmitter ratios were less than ten percent, allowing for implantation. Considerably fewer adult female *A. maculatum* were captured at site 26031. All salamanders selected for implantation met the criteria of the linear regression model and the body weight to transmitter weight ratio.

Surgical Implantation

Eighteen salamanders (site 26007 n = 11, site 26031 n = 7) had radio transmitters surgically implanted. No salamanders perished nor were abnormal behaviors observed as a result of the surgical procedure. The times to induction, length of surgery, and time to initial and full recovery are summarized in Table 3. These times are considered sufficient and reasonable when compared to protocols established by the United States Geological Survey (n.d). All surgeries were performed the day following

26007							
Name	Quadrant	Pitfall No.	Weight (g)	SVL (mm)	TL (mm)	Transmitter Type	Transmitter Frequency
Ryland	1	6	30.6	115.0	225.0	BD-2HX	149.470
Sam	1	8	32.9	114.0	222.0	BD-2HX	149.097
Emma	1	34	31.4	112.0	216.0	PD-2X	149.216
Gabba	2	10	27.8	108.0	203.0	BD-2HX	149.722
Lauren	2	12	31.3	109.0	224.0	BD-2HX	149.351
Elizabeth	2	14	26.5	110.0	217.0	BD-2HX	149.650
Macey	3	18	31.7	114.0	218.0	PD-2X	149.148
Carlise	3	20	32.2	104.0	225.0	PD-2X	149.118
Hannah	3	22	30.2	112.0	217.0	BD-2HX	149.550
Zoe	4	30	40.6	122.0	245.0	PD-2X	149.067
Sandie	4	32	31.1	119.0	226.0	BD-2HX	149.256

26031							
Name	Quadrant	Pitfall No.	Weight (g)	SVL (mm)	TL (mm)	Transmitter Type	Transmitter Frequency
Lindsey	1	6	29.5	108.0	198.0	BD-2HX	149.950
Ashley	2	10	24.6	99.0	196.0	BD-2HX	149.911
Daisy	2	12	27.4	111.0	221.0	PD-2X	149.297
Olivia	2	14	24.3	96.0	199.0	BD-2HX	149.770
Kameron	3	16	27.2	116.0	213.0	BD-2HX	149.870
Jayda	3	18	25.0	106.0	204.0	BD-2HX	149.377
Megan	4	2	25.1	112.0	214.0	BD-2HX	149.810

Table 2. Morphological and radiotransmitter data for *A. maculatum* selected for implantation.

Name	Anesthesia			Surgery			Recovery		
	Start	Induction		Start	Stop	Start	Initial	Full	
26007									
Zoe	1223	1312		1312	1340	1340	1412	1412	
Sam	1252	1346		1346	1402	1402	1428	1456	
Carlise	1312	1345		1404	1416	1416	1536	1605	
Emma	1424	1520		1525	1536	1536	1555	1656	
Macey	1440	1510		1523	1523	1523	1536	1607	
Sandie	1526	1608		1608	1621	1715	1744	1744	
Lauren	1540	1642		1642	1654	1654	1712	1720	
Ryland	1608	1659		1700	1711	1711	1745	1828	
Hannah	1657	1745		1745	1755	1755	1828	1910	
Elizabeth	1713	1805		1805	1815	1815	1917	2040	
Gabba	1757	1910		1910	1921	1921	2041	2055	
Average:			0:50	:12			:38	1:09	
26031									
Name	Anesthesia			Surgery			Recovery		
	Start	Induction		Start	Stop	Start	Initial	Full	
Lindsey	754	850		851	906	906	1024	1034	
Jayda	822	908		909	920	920	1125	1153	
Daisy	846	947		947	1002	1002	1037	1111	
Kameron	901	1000		1005	1019	1019	1050	1125	
Megan	924	1040		1040	1054	1054	1102	1125	
Olivia	1004	1112		1112	1124	1124	1156	1234	
Ashley	1029	1128		1128	1043	1043	1245	1319	
Average:			1:00	:13			1:01	1:30	

Table 3. A summary of the times of induction, surgery, initial recovery, and full recovery for all implantation surgeries.

collection and released a minimum of 24 hours following full recovery at the point of capture

Typically, sutures remain in place until they are removed after the healing process has occurred. In most telemetry studies, sutures remain in place until the research project has reached its completion and the animals can be retrieved at which time sutures can be removed and transmitters recovered. External sutures that remain in place throughout a project may present issues to animals that spend a majority of their time in subterranean retreats. Sutures or transmitters may get caught on habitat features during movements, likely causing trauma or injury to the animal. Madison (1997) observed an *A. maculatum* entangled in a root mass as the result of the implant itself. Based on this observation, sutures may lead to indirect mortalities of salamanders. Vicryl® sutures are ones that dissolve throughout the healing process, and once healing is complete, there is no need to capture and remove the sutures post healing. Using this type of suture may eliminate potential trauma and injury to research subjects.

The 0 Vicryl® sutures were likely too large to use with *A. maculatum*, but this type of suture was the only size and brand available at the time of the research. Figure 36 shows the size of the suture used on *A. maculatum* to close the incision during surgery. Furthermore, the size of the sutures made it difficult to minimize the size of the knot. This was the only problem that arose from using this size of suture and possibly made the use of subterranean burrows difficult.

Approximately three weeks into the telemetry, two salamanders were excavated to verify the health of the animals and to ensure the radio transmitters had not been lost. Surprisingly, the Vicryl® sutures had already disappeared and there was little evidence



Figure 36. A photograph of a sutured incision of an implanted *A. maculatum* following surgery.

of a surgical procedure. Figure 37 shows a salamander that was excavated at the end of the study. The use of dissolvable stitches may eliminate potential injury resulting from salamanders retaining sutures throughout a research project or its life in the event they are not captured upon a research project ending. It does not appear the salamanders in this study suffered any effects from the dissolving sutures; however, potential impacts from the use of this type of suture may need further investigation.

Radiotracking

Eighteen *A. maculatum* were radio tracked from 03 March 2011 through 09 June 2011, the conclusion of the project. Eleven salamanders from site 26007 and seven from site 26031 were implanted with radio transmitters. One hundred and sixty four total locations were made during the three months of radio tracking, and individual salamanders were located between 5 – 12 times each ($\bar{x} = 9.1$, min = 5, max = 12). Although the final locations of all salamanders were not determined, it is believed only one *A. maculatum* was preyed on during telemetry. The specific identity of the predator is unknown because only the radio transmitter was found.

Numerous migration events were observed at both sites during the 98 days of radio-tracking. *Ambystoma maculatum* traveled a maximum straight-line distance of 840 m from site 26007 ($\bar{x} = 388.18\text{m}$ / salamander, n = 11, range = 80 to 840 m) and 480 m from site 26031 ($\bar{x} = 310.67\text{m}$ / salamander, n = 7, range = 94 to 480 m). Migration distances and the final bearings of travel from each site are summarized in Table 4. Maps depicting the migrations and final locations can be found in Appendix 4.



Figure 37. A salamander excavated upon the conclusion of the study showed no physical effects from the use of a dissolvable suture.

ID	Mass (g)	Days	Fixes	Fate	TD (m)	MD (m)	SD (m)	Bearing
26007								
Ryland	30.6	97	10	A	375	310	340	166
Sam	32.9	97	10	A	843	230	840	68
Gabba	27.8	97	7	A	660	600	640	49
Lauren	31.3	97	10	A	91	58	110	310
Elizabeth	26.5	42	8	U	227	220	220	342
Macey	31.7	97	11	A	634	250	600	48
Carlise	32.2	57	8	U	622	200	580	313
Sandie	31.1	57	11	U	273	140	220	62
Emma	31.4	27	6	P	90	80	80	341
Hannah	30.2	57	11	U	144	74	140	325
Zoe	40.6	97	10	A	620	190	500	344
26031								
Megan	25.1	98	12	A	267	120	220	184
Lindsey	29.5	31	5	U	422	410*	410*	5
Ashley	24.6	58	10	U	482	210	480	28
Daisy	27.4	32	9	U	361	130	350	52
Olivia	24.3	32	9	U	94	94	94	340
Kameron	27.2	98	9	A	436	380	360	287
Jayda	25.0	31	8	U	360	190	360	284

Days = the number of days individual salamanders were tracked; **Fixes** = the number of times salamanders were located during the study; **Fate**: A = Alive, P = Predated, U = Unknown; **TD** = the total distance salamanders traveled; **MD** = maximum distance traveled during a single migration event, **SD** = maximum overall distance traveled from the point of release at the breeding site; **Bearing** = the bearing of travel from the breeding site to the final location

* Three weeks passed between first location, MD and SD. It is unknown if MD and SD occurred in a single night due to lack of fixes for this salamander.

Table 4. Migration distances and bearing of travel observed at sites 26007 and 26031.

Discussion

The average migration distances observed in this study, 388.18 m (26007) and 310.67 m (26031) are the longest ever reported for *A. maculatum*. Wacasey (1961) determined *A. maculatum* migrated an average of 66 m by conducting terrestrial searches in Michigan. Williams (1970) tracked *A. maculatum* with implanted tantalum¹⁸² wire and found the species migrated an average of 64.2 m from wetlands. In Michigan, Kleeberger and Werner (1983) implanted *A. maculatum* with cobalt⁶⁰ wire tags and found migration distances averaged 192 m. Madison (1997) implanted *A. maculatum* in New York across multiple years with radio transmitters and migration distances averaged 42.3 m and 118.0 m. Average migration distances of 93.8 m (Bailey and Bailey 2000; Tennessee), 101.1 m (Faccio 2003; Maine), 145.0 m (Montieth and Patton 2006; Rhode Island), and 62.5 m, 89.4 m, 102.3m, and 213.9 m (McDonough and Paton 2007; Connecticut) using the techniques of Madison (1997) have been reported. Prior to this study, the longest straight-line migration from a wetland by *A. maculatum* was 467.0 m (McDonough and Patton 2007). A single *A. maculatum* tracked during this study migrated 840 m from the breeding site. The variation in migratory distances for this species increases the difficulty in determining the appropriate conservation and management strategies to implement.

Semlitsch (1998) proposed the application of terrestrial “buffer zones” to protect terrestrial habitat surrounding wetlands through the use of the following formula taken from Bailey and Bailey (2000):

$$95 \text{ percent confidence limits} = \text{mean distance} \pm (t_{[\alpha=0.05(2)]} * s / n^{1/2}).$$

Using the mean migration distances observed in this study, the terrestrial buffer at each site necessary to protect 95% of the breeding population is 557.89 m (97.78ha) at site 26007 and 439.27 m (60.62 ha) at site 26031. These buffer zones, if applied, represent vast amounts of unmanageable land allowing the protection of terrestrial habitat critical for *A. maculatum* outside the breeding season. This buffer zone would also allow for the development of juvenile amphibians critical for the persistence of populations.

Tremendous variation has been observed in the migration distances of *A. maculatum* geographically. Both buffer zones calculated by Semlitsch (1998) and Bailey and Bailey (2000) were considerably smaller than those necessary to protect both breeding populations of *A. maculatum* on Bear Hollow Mountain WMA. Bailey and Bailey (2000) determined the size of the necessary buffer zone to be considerably less (131.1m), but noted wetlands suitable for breeding were not uncommon across the study site. Few wetlands on Bear Hollow Mountain WMA exist that are suitable for breeding by *A. maculatum* (Figure 3). Given much of the WMA is suitable habitat for *A. maculatum*, it is unlikely the species limits its range on the WMA to those areas immediately surrounding likely breeding sites. Most regulations and best management practices (BMPs) applied to wetlands focus on the protection of water quality through implementation of management to minimize degradation of terrestrial habitat surrounding wetlands. Tennessee Department of Agriculture (2003) summarizes the nonregulatory BMPs to prevent nonpoint source pollution. There are numerous BMPs prescribed to minimize the flow of fill materials into wetlands and the only buffers recommended are those that fall under the guidelines of stream management zones (SMZs). SMZs must be created a minimum of 7.62 meters from the edge of water, the width of which increases

with elevation of the site. Up to fifty percent of the canopy can be removed within the SMZ to maintain ample canopy cover to provide shade to the water (Tennessee Department of Forestry pers. comm). These nonregulatory practices do not focus on the protection of terrestrial habitat necessary to protect amphibian populations.

Gamble *et al.* (2006) determined regulations used to protect upland habitat surrounding wetland habitats were insufficient because salamanders migrated outside of buffers following breeding. Semlitsch and Bodie (2003) revised the criteria for terrestrial buffers to include an aquatic buffer immediately surrounding the wetland (30-60 m), a core habitat (142-289 m) and a terrestrial buffer surrounding both the aquatic buffer and core habitat (50 m). Although his revision seeks to protect both the aquatic and terrestrial habitat, these recommendations, if applied to wetlands at Bear Hollow Mountain WMA, still would be insufficient in protecting the necessary core habitat for amphibians.

Application of buffers for the protection of wetlands and terrestrial habitats must take into consideration factors beyond life history requirements of species. Although the terrestrial habitat surrounding 26007 was unaltered and continuous, *A. maculatum* migrations were considerably greater than those observed at site 26031, which has undergone tremendous alteration. Most telemetered *A. maculatum* moved to locations down slope from breeding sites even though suitable terrestrial habitat was present in the vicinity of site 26007 and site 26031. Possibly, this presumably suitable terrestrial habitat was not preferred by the species, which caused their down-slope migration. The upland terrestrial habitat may lack numerous key components, such as moisture and temperature regimes and ample subterranean burrows preferred by the species. Habitats located down slopes appeared to have deeper soils, thicker detritus layers, rock, and more

subterranean burrows that likely allow these areas to act as refugia for *A. maculatum* during the nonbreeding portions of the year; however, characteristics of these habitats need further investigation.

Ambystoma maculatum migrated across the clearcut surrounding site 26031, portions of which were completely void of vegetation due to current habitat management. Migrations across open habitats have been observed in the species (Montieth and Paton 2006; Veysey *et al.* 2009) indicating open areas do not represent barriers to dispersal. The habitat manipulation at site 26031 did not increase migration distance or selection of available upland habitat around the site as hypothesized when compared to those observed at 26007. Possibly, the migration distances observed at site 26031 were shorter due to its proximity to habitats down slope compared to the proximity of site 26007 to similar down slope habitat.

The use of buffers to protect upland habitat for amphibians is necessary, but application of standard-sized buffers may be inadequate and unnecessary. Protecting the amount of upland necessary to protect 95% of the population would decrease land managers' ability to develop and maintain habitat for other species of wildlife. If an average buffer size of 80.94 ha were placed around wetlands on Bear Hollow Mountain WMA, less than 10% of the WMA would become unmanageable, potentially decreasing the ability of TWRA to create habitat for other species. Because most telemetered *A. maculatum* moved downslope, precautions should be taken to protect these areas for the species decreasing the potential amount of unmanageable land. In the case of site 26031, TWRA should consider altering its current habitat management to minimize any negative

impacts to juvenile amphibians because habitat work is occurring such close proximity to the breeding site.

In conclusion, SMZs recommended by the Tennessee Department of Agriculture are insufficient in protecting water quality and upland terrestrial habitat around wetlands. TWRA should seek to apply buffers considerably larger than those recommended in the event habitat management is necessary in areas surrounding wetlands. It is unknown as to how large a buffer should be used at wetlands on Bear Hollow Mountain and further studies should seek to determine the amount of terrestrial habitat necessary for the maintenance of emigrating juvenile salamanders. Furthermore, buffers should be considerably larger than 7.62 meters from the edge of the water and no timber harvesting should be allowed within these zones.

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Appendices

Appendix 1:

Avian Data

Bird Species heard or observed at sites 26007 and 26031. P = Species was present, FO =
Flyover

Species	Site	
	26007	26031
Wood Duck		P
Sandhill Crane	FO	
Mourning Dove		P
Red-bellied Woodpecker		P
Northern Flicker		P
Pileated Woodpecker	P	P
Yellow-billed Cuckoo	P	
Eastern Wood-pewee	P	P
White-eyed Vireo	P	P
Red-eyed Vireo	P	P
Blue Jay	P	P
Carolina Chickadee	P	
Tufted Titmouse	P	
White-breasted Nuthatch	P	
Carolina Wren	P	P
Blue-gray Gnatcatcher	P	
Veery	P	
Wood Thrush	P	
Blue-winged Warbler		P
Northern Parula	P	
Black-throated Green Warbler	P	
Black and White Warbler	P	P
Prairie Warbler		P
Kentucky Warbler	P	
Yellow-breasted Chat		P
Scarlet Tanager	P	P
Eastern Towhee	P	P
Chipping Sparrow	P	P
Field Sparrow		P
Northern Cardinal		P
Indigo Bunting		P
Brown-headed Cowbird		P
American Goldfinch		P
Eastern Wild Turkey	P	P

Appendix 2:

Species Lists for Each Site

Total captures of animals entering and exiting site 26007. Imm = immgrants, Emi = emigrants, Tot = total captured, * = denotes species of greatest conservation need; ¹ = identification of species pending

Species	Common Name	Imm	Emi	Tot
Bufonidae				
<i>Anaxyrus americanus</i>	American Toad	87	50	137
<i>Anaxyrus fowleri</i>	Fowler's Toad	1	2	3
Hylidae				
<i>Acris crepitans</i>	Northern Cricket Frog	6	4	10
<i>Hyla gratiosa</i> *	Barking Treefrog	1	0	1
<i>Hyla versicolor</i>	Gray Treefrog	44	3	47
<i>Pseudacris crucifer</i>	Northern Spring Peeper	670	81	751
<i>Pseudacris feriarum</i>	Upland Chorus Frog	0	1	1
Microhylidae				
<i>Gastrophryne carolinensis</i>	Eastern Narrowmouth Toad	73	37	110
Ranidae				
<i>Lithobates catesbeiana</i>	Bullfrog	11	3	14
<i>Lithobates clamitans</i>	Green Frog	71	34	105
<i>Lithobates palustris</i>	Pickerel Frog	16	11	217
<i>Lithobates utricularius</i>	Southern Leopard Frog	58	46	104
Scaphiopodidae				
<i>Scaphiopus holbrooki</i>	Eastern Spadefoot	181	170	351
Ambystomatidae				
<i>Ambystoma maculatum</i>	Spotted Salamander	5826	3716	9542
<i>Ambystoma opacum</i>	Marbled Salamander	3673	5718	9391
<i>Ambystoma talpoideum</i>	Mole Salamander	1941	2187	4128
<i>Ambystoma tigrinum</i>	Eastern Tiger Salamander	2	2	4
Plethodontidae				
<i>Hemidactylium scutatum</i> *	Four-toed Salamander	238	65	303
<i>Plethodon dorsalis</i>	Northern Zigzag Salamander	23	6	29
<i>Plethodon glutinosus</i>	Northern Slimy Salamander	15	1	16
Salamandridae				
<i>Notophthalmus viridescens</i>	Eastern Newt	1464	2029	3493
Chelydridae				
<i>Chelydra serpentina</i>	Common Snapping Turtle	13	5	18
Emydidae				
<i>Terrapene carolina</i> *	Eastern Box Turtle	1	1	2
Kinosternidae				
<i>Kinosternon subrubrum</i>	Eastern Mud Turtle	3	0	3
Scincidae				
<i>Plestiodon laticeps</i>	Broadhead Skink	0	1	1
Colubridae				
<i>Carphophis amoenus</i>	Eastern Worm Snake	9	8	17
<i>Coluber constrictor</i>	Eastern Racer	21	13	34
<i>Lampropeltis triangulum</i>	Milk Snake	0	1	1

<i>Pantherophis spiloides</i>	Rat Snake	1	0	1
Crotalidae				
<i>Agkistrodon contortrix</i>	Copperhead	44	21	65
<i>Crotalus horridus</i> *	Timber Rattlesnake	2	1	3
Dipsadidae				
<i>Diapophis punctatus</i>	Ringneck Snake	1	2	3
Natricidae				
<i>Nerodia sipedon</i>	Northern Water Snake	12	7	19
<i>Storeria occipitomaculata</i>	Red-bellied Snake	1	1	2
<i>Thamnophis sirtalis</i>	Common Garter Snake	2	4	6
<i>Virginia valeriae</i>	Smooth Earthsnake	1	1	2
Cricetidae				
<i>Neotoma floridana</i> *	Eastern Woodrat	1	1	2
<i>Reithrodontomys humilis</i>	Eastern Harvest Mouse	1	0	1
Sciuridae				
<i>Glaucomys volans</i>	Southern Flying Squirrel	0	1	1
<i>Microtus ochrogaster</i>	Prairie Vole	0	1	1
<i>Microtus pinetorium</i>	Woodland Vole	16	5	21
<i>Peromyscus leucopus</i>	White-footed Mouse	8	5	13
<i>Peromyscus maniculatus</i>	Deer Mouse	1	1	2
<i>Sigmodon hispidus</i>	Hispid Cotton Rat	2	2	0
Soricidae				
<i>Blarina brevicauda</i>	Northern Short-tailed Shrew	21	4	25
<i>Sorex hoyii</i> *	Pygmy Shrew	1	0	1
<i>Sorex longirostris</i> * ^l	Southeastern Shrew	1	1	2
	Total	14,463	14,252	28,815

Total captures of animals entering and exiting site 26031 (Imm = immgrants, Emi = emigrants, tot = total, * denotes species of greatest conservation need).

Species	Common Name	Imm	Emi	Tot
Bufonidae				
<i>Anaxyrus americanus</i>	American Toad	608	504	1112
<i>Anaxyrus fowleri</i>	Fowler's Toad	3	4	7
Hylidae				
<i>Hyla versicolor</i>	Gray Treefrog	12	4	16
<i>Pseudacris brachyphona</i> *	Mountain Chorus Frog	0	1	1
<i>Pseudacris crucifer</i>	Northern Spring Peeper	51	17	68
<i>Pseudacris feriarum</i>	Upland Chorus Frog	1	0	1
Microhylidae				
<i>Gastrophryne carolinensis</i>	Eastern Narrowmouth Toad	118	39	157
Ranidae				
<i>Lithobates clamitans</i>	Green Frog	14	6	20
<i>Lithobates utricularius</i>	Southern Leopard Frog	35	25	60
Scaphiopodidae				
<i>Scaphiopus holbrookii</i>	Eastern Spadefoot	77	81	158
Ambystomatidae				
<i>Ambystoma maculatum</i>	Spotted Salamander	973	919	1892
<i>Ambystoma opacum</i>	Marbled Salamander	709	2979	3688
<i>Ambystoma talpoideum</i>	Mole Salamander	664	1004	1668
<i>Ambystoma tigrinum</i>	Eastern Tiger Salamander	1	2	3
Plethodontidae				
<i>Hemidactylium scutatum</i> *	Four-toed Salamander	43	15	58
<i>Plethodon dorsalis</i>	Northern Zigzag Salamander	15	23	38
<i>Plethodon glutinosus</i>	Northern Slimy Salamander	0	1	1
<i>Pseudotriton ruber</i>	Red Salamander	1	0	1
Salamandridae				
<i>Notophthalmus viridescens</i>	Eastern Newt	60	96	156
Phrynosomatidae				
<i>Sceloporus undulatus</i>	Eastern Fence Lizard	3	2	5
Scincidae				
<i>Plestiodon inexpectatus</i>	Southeastern Five-lined Skink	1	1	2
<i>Plestiodon laticeps</i>	Broadhead Skink	3	8	11
Colubridae				
<i>Carphophis amoenus</i>	Eastern Worm Snake	2	1	3
<i>Coluber constrictor</i>	Eastern Racer	15	10	25
<i>Pantherophis spiloides</i>	Rat Snake	2	4	6
Crotalidae				
<i>Agkistrodon contortrix</i>	Copperhead	7	6	13
<i>Crotalus horridus</i> *	Timber Rattlesnake	2	1	3
Natricidae				
<i>Nerodia sipedon</i>	Northern Water Snake	1	0	1
<i>Storeria occipitomaculata</i>	Red-bellied Snake	0	1	1
<i>Thamnophis sirtalis</i>	Common Garter Snake	8	10	18

Xenodontidae				
<i>Heterodon platirhinos</i> *	Eastern Hognose Snake	4	2	6
Cricetidae				
<i>Microtus ochrogaster</i>	Prairie Vole	6	2	8
<i>Microtus pinetorium</i>	Woodland Vole	1	0	1
<i>Ochrotomys nuttali</i> *	Golden Mouse	1	0	1
<i>Peromyscus leucopus</i>	White-footed Mouse	7	5	12
<i>Peromyscus maniculatus</i>	Deer Mouse	3	1	4
<i>Sigmodon hispidus</i>	Hispid Cotton Rat	1	0	1
Sciuridae				
<i>Tamias striatus</i>	Eastern Chipmunk	1	1	2
Soricidae				
<i>Blarina brevicauda</i>	Northern Short-tailed Shrew	8	4	12
<i>Cryptotis parva</i>	Least Shrew	5	1	6
<i>Sorex longirostris</i>	Southeastern Shrew	1	1	2
	Total	3,467	5,781	9,248

Appendix 3:

Results of Vector Analysis

Table A3-1. Mean vector of movement across all years for *A. opacum* at site 26007. (F = Immigrating Females, M = Immigrating Males, E = Emigrating Adults, J = Emigrating Juveniles, and O = Overall). Analyses were not conducted for first year adult third year juvenile movements because of the survey dates.

	Year 1			Year 2			Year 3				
	J	F	M	E	J	O	F	M	E	J	O
N	3438	1428	1274	581	611	2943	195	71	249	-	283
Vector (°)	261.8	239.5	267.5	281.1	247.6	236.2	272.5	86.8	240.8	-	267.3
Circular SD (°)	112.3	91.3	128.9	87	78.4	107.9	110.6	96.9	85.4	-	128.3
SE of (°)	1.9	2.4	3.6	3.6	3.2	1.9	7.9	11.5	0.18	-	7.6
Rao's Spacing Test (U)	357.3	353.9	352.9	345.7	351.1	356.9	324.9	284.6	333.9	-	333.2
Rao's Spacing Test (<i>p</i>)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01

Table A3-2. Mean vector of movement across all years for *A. opacum* at site 26031. (F = Immigrating Females, M = Immigrating Males, E = Emigrating Adults, J = Emigrating Juveniles, and O = Overall). Analyses were not conducted for first year adult third year juvenile movements because of the survey dates.

	Year 1			Year 2			Year 3				
	J	F	M	E	J	O	F	M	E	J	O
N	1699	331	70	121	644	415	75	33	72	-	119
Vector (°)	64.1	266	246.1	232.4	263.2	259.4	163.3	129.3	305.6	-	161.7
Circular SD (°)	98.8	89.7	86.1	81.8	93.9	89.9	103.1	89.2	101.3	-	100.1
SE of (°)	2.4	4.9	10.3	7.4	3.7	4.4	11.9	15.5	11.9	-	9.2
Rao's Spacing Test (U)	356.3	341.5	288	321.3	352.7	345.2	302.4	240	300	-	323.6
Rao's Spacing Test (<i>p</i>)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01

Table A3-3. Mean vector of movement across all years for *A. maculatum* at site 26007. (F = Immigrating Females, M = Immigrating Males, E = Emigrating Adults, J = Emigrating Juveniles, and O = Overall). Analyses were not conducted for first year adult third year juvenile movements because of the survey dates.

	Year 1					Year 2					Year 3				
	F	M	E	J	O	F	M	E	J	O	F	M	E	J	O
N	789	2769	1140	470	3689	321	611	586	15	938	369	753	296	-	1147
\bar{x} Vector (°)	64.9	60.2	291.7	248.6	55.9	89.5	86.3	296.3	281.9	87.5	126.9	87.4	269.5	-	97.5
Circular SD (°)	95.7	94.1	69.8	110.1	96.7	96.3	80.3	75.9	70.5	85.6	120.2	95.3	79.9	-	100.9
SE of \bar{x} (°)	3.4	1.8	2.1	5.1	1.6	5.3	3.3	3.1	18.2	2.8	5.3	3.5	4.6	-	2.9
Rao's Spacing Test (U)	351.7	357.2	352.4	340.8	357.7	338.6	347.6	347.7	194.7	350.7	342.4	350.4	335.6	-	353.7
Rao's Spacing Test (<i>p</i>)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01

Table A3-4. Mean vector of movement across all years for *A. maculatum* at site 26031. (F = Immigrating Females, M = Immigrating Males, E = Emigrating Adults, J = Emigrating Juveniles, and O = Overall). Analyses were not conducted for first year adult third year juvenile movements because of the survey dates.

	Year 1					Year 2					Year 3				
	F	M	E	J	O	F	M	E	J	O	F	M	E	J	O
N	235	316	315	102	564	84	99	112	16	183	99	98	127	-	199
\bar{x} Vector (°)	345.2	334.1	29.9	345.1	339.6	344.1	359.1	351.3	43.3	351.4	7.8	11.6	339.3	-	8.5
Circular SD (°)	72.5	87	105.6	123.5	81.5	74.9	84.5	105.4	71.7	80.4	69.3	83.5	97.3	-	77.1
SE of \bar{x} (°)	4.7	4.9	5.9	12.2	3.4	8.2	8.5	9.9	17.9	5.9	6.9	8.4	8.6	-	5.5
Rao's Spacing Test (U)	337.0	344.0	341.7	307.0	349.7	312.8	312.7	311.7	218.3	334.4	312.7	312.2	320.3	-	334.6
Rao's Spacing Test (<i>p</i>)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01

Table A3-5. Mean vector of movement across all years for *A. talpoideum* at site 26007. (F = Immigrating Females, M = Immigrating Males, E = Emigrating Adults, J = Emigrating Juveniles, and O = Overall). Analyses were not conducted for third year juveniles because of survey dates.

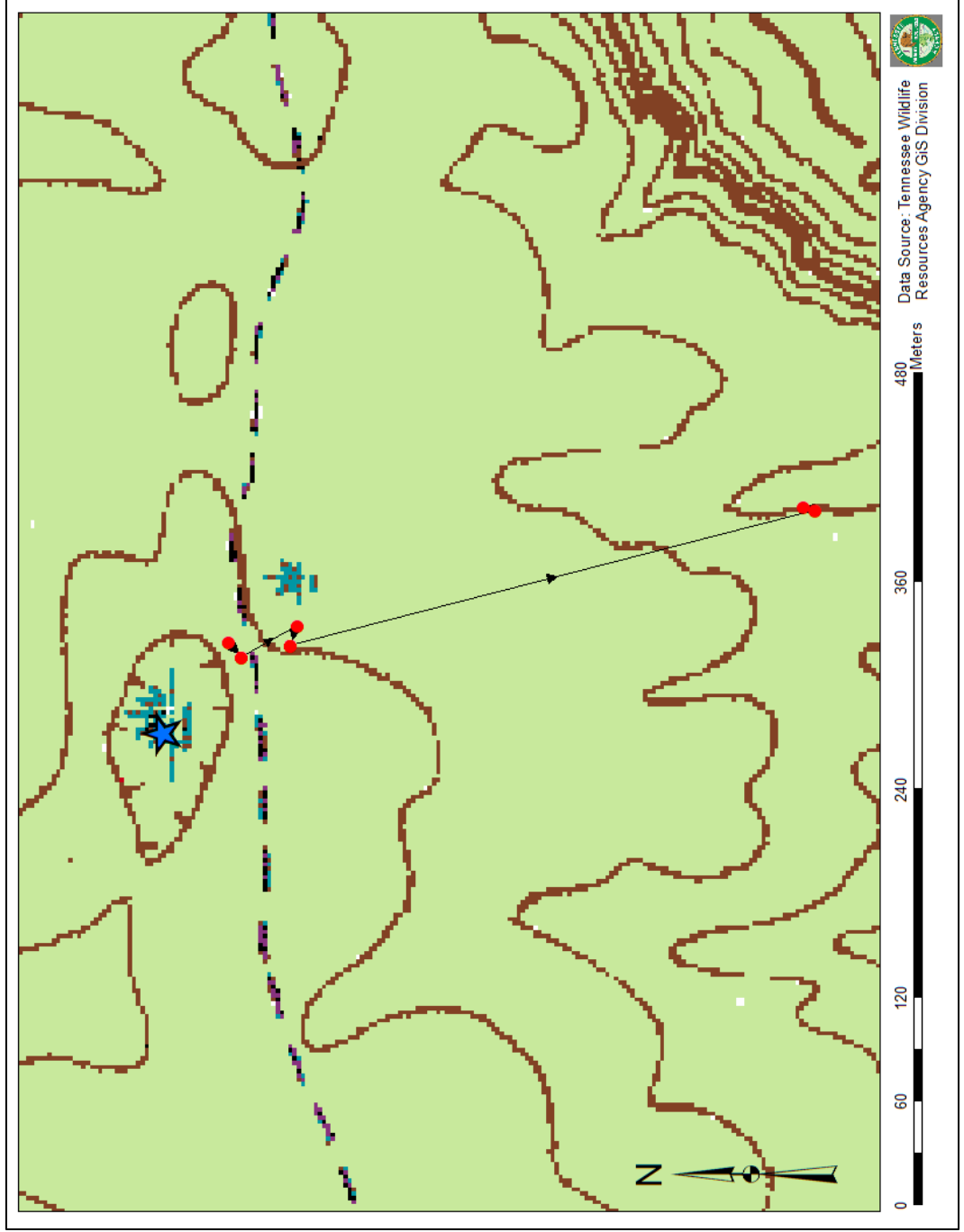
	Year 1					Year 2					Year 3				
	F	M	E	J	O	F	M	E	J	O	F	M	E	J	O
N	399	749	737	487	1204	176	229	379	257	415	76	132	253	-	213
\bar{x} Vector (°)	80.4	70.9	262.5	162.1	71.1	132.6	145.1	281.9	152.5	138.9	65.4	66	270.1	-	67.6
Circular SD (°)	102.7	91.8	86.1	116.7	97	83.8	84.5	69.9	127.5	86.1	81.5	108.9	72.3	-	97.6
SE of \bar{x} (°)	5.1	3.4	3.2	5.3	2.8	6.3	5.6	3.6	30.0	7.1	9.3	9.5	4.5	-	6.7
Rao's Spacing Test (U)	342.8	348.4	348.2	341.5	352.5	325.2	331.7	342.9	357.4	343.5	284.5	310.9	331.5	-	327.8
Rao's Spacing Test (<i>p</i>)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-	<0.01

Table A3-6. Mean vector of movement across all years for *A. talpoideum* at site 26031. (F = Immigrating Females, M = Immigrating Males, E = Emigrating Adults, J = Emigrating Juveniles, and O = Overall). Analyses were not conducted for third year juveniles because of survey dates.

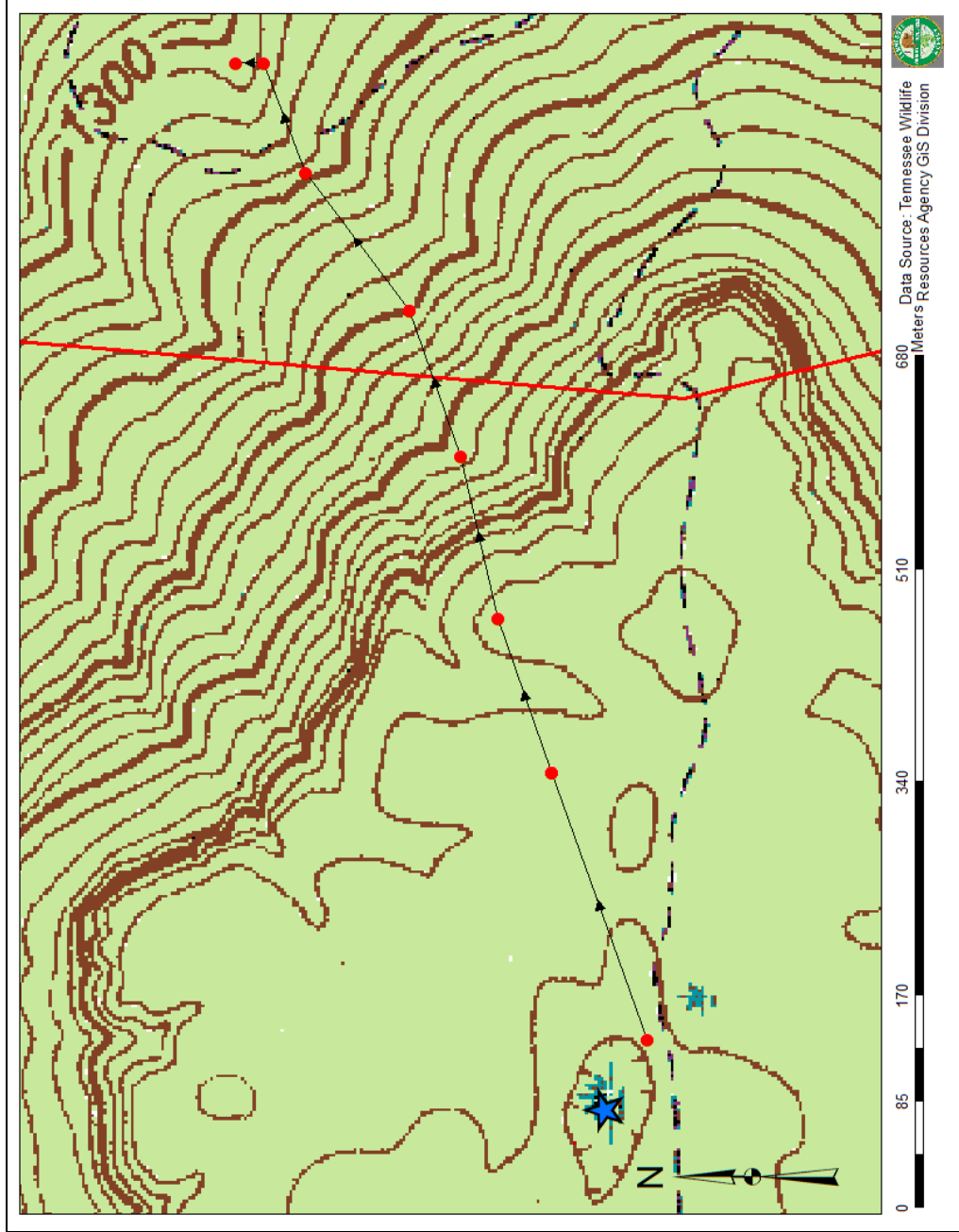
	Year 1					Year 2					Year 3				
	F	M	E	J	O	F	M	E	J	O	F	M	E	J	O
N	239	179	438	333	466	47	42	129	5	89	43	38	85	-	82
\bar{x} Vector (°)	297.6	312.5	164.5	102.8	304.6	342.5	248.7	218.4	152.9	291.7	325.6	192.7	94.1	-	295.6
Circular SD (°)	103.5	104	113.7	90.3	104.8	95.4	86.7	105.7	43.3	103.9	104	118.9	130.9	-	134.6
SE of \bar{x} (°)	6	7.8	5.4	4.9	4.9	13.9	13.4	9.3	19.4	11	15.9	19.3	14.2	-	14.9
Rao's Spacing Test (U)	340.4	327.8	346.0	343.7	346.8	291.0	274.2	326.5	188.1	311.4	293.0	265.2	304.9	-	311.7
Rao's Spacing Test (<i>p</i>)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	-	<0.01

Appendix 4:
Telemetry Locations

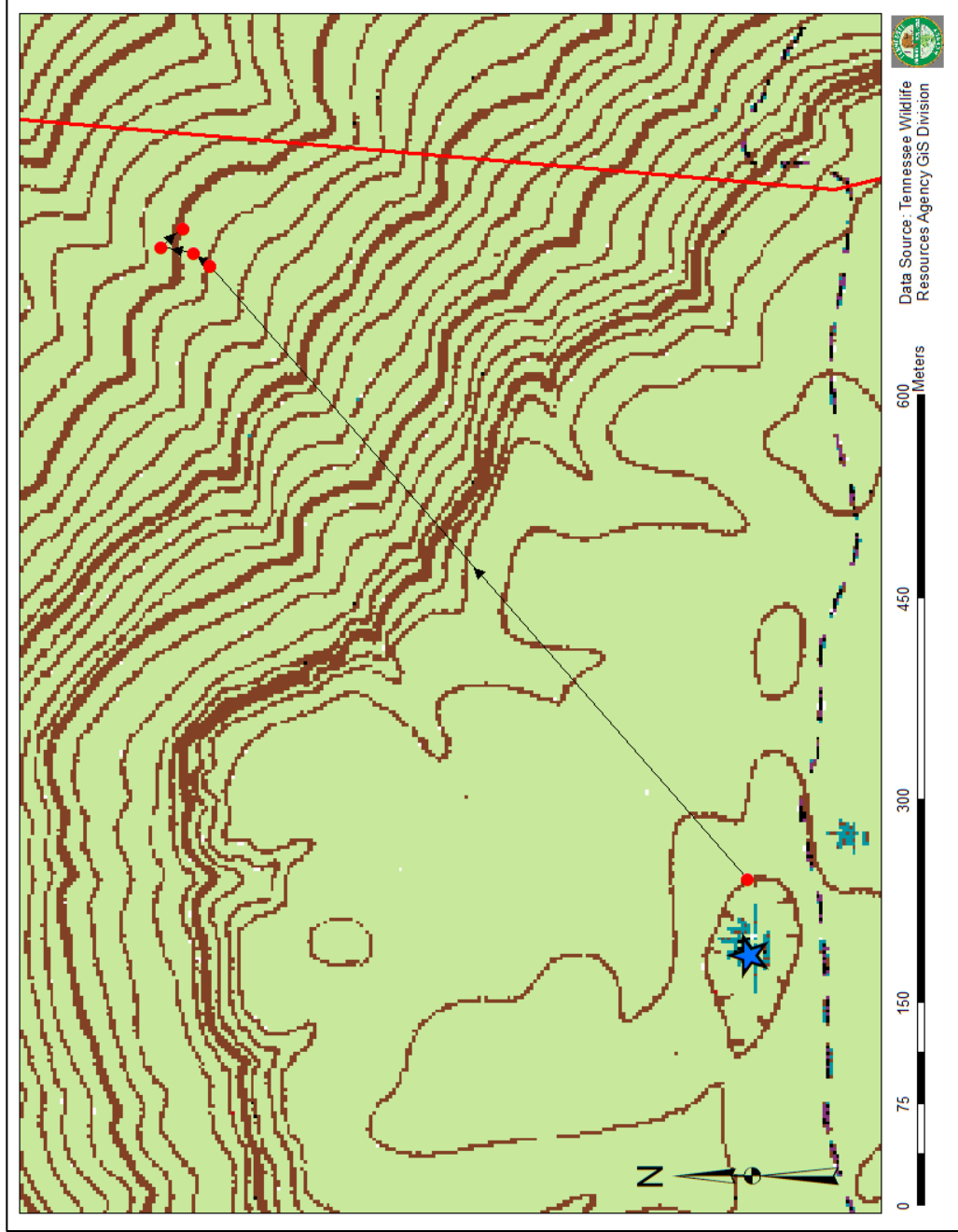
26007 – Ryland. Straight-line distance moved from point of release was 340 m.



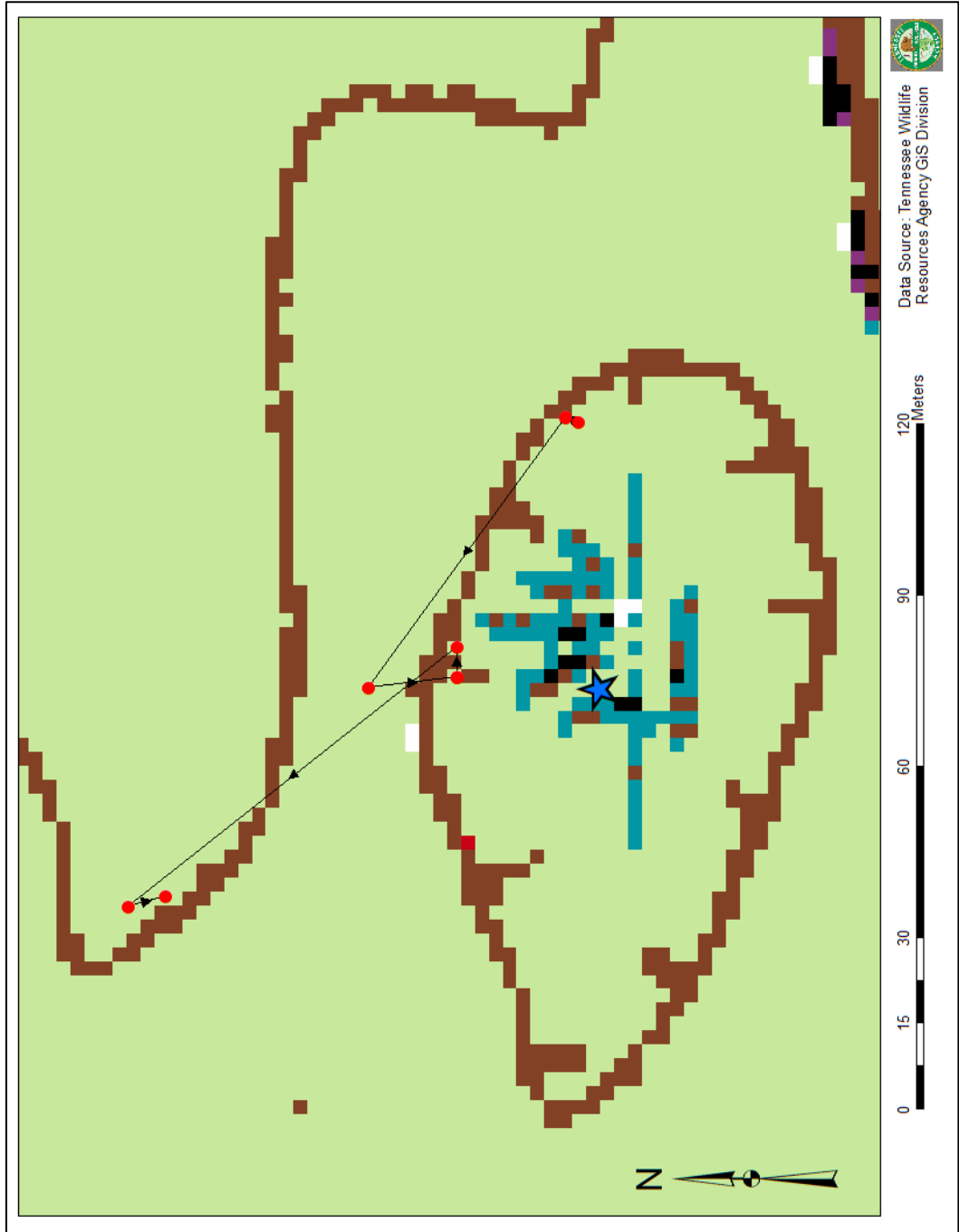
26007- Sam. Straight-line distance moved from the point of release was 840 m. The red line indicates the eastern boundary of the WMA.



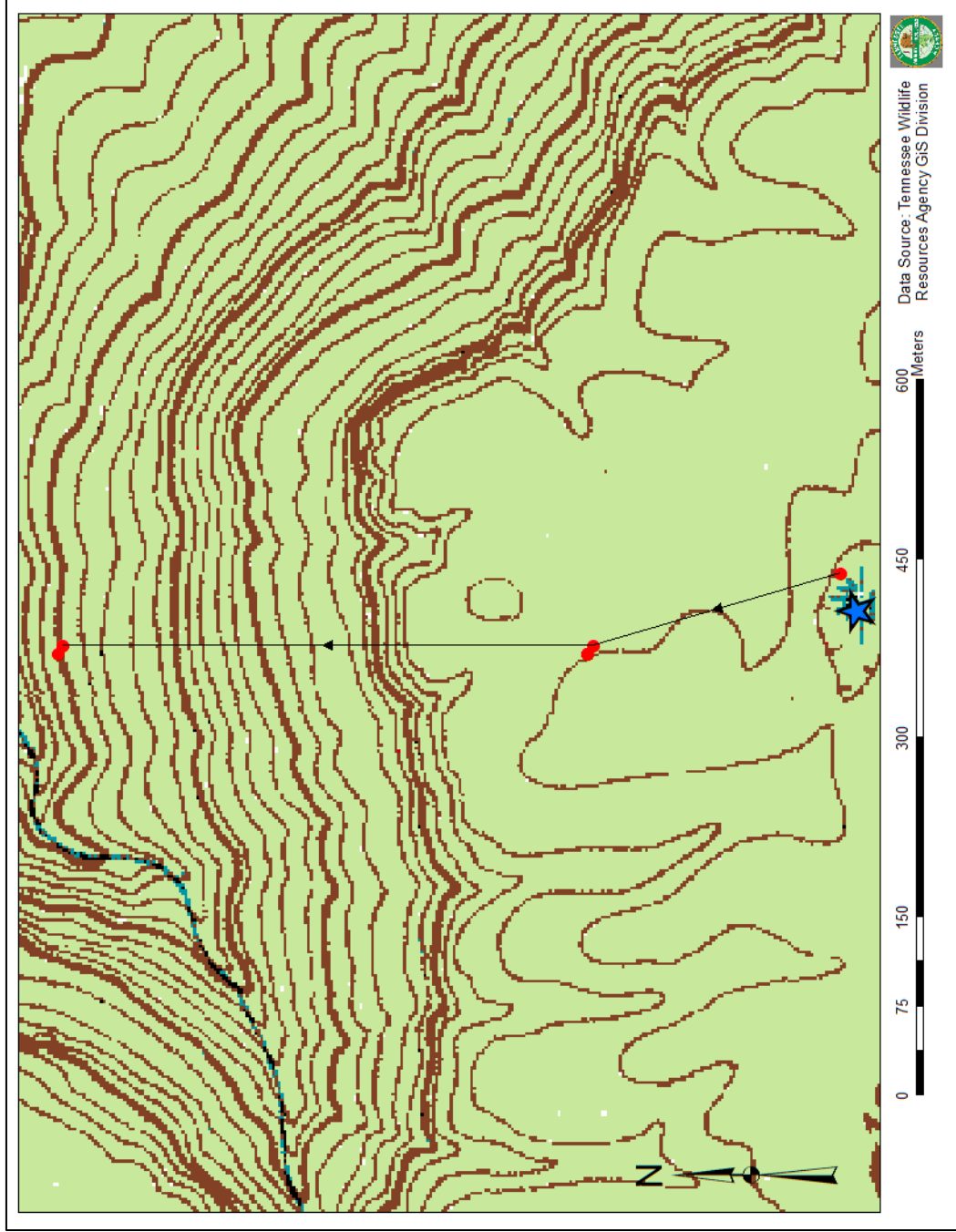
26007 – Gabba. Straight-line distance moved from the point of release was 640 m. The red line indicates the eastern boundary of the WMA.



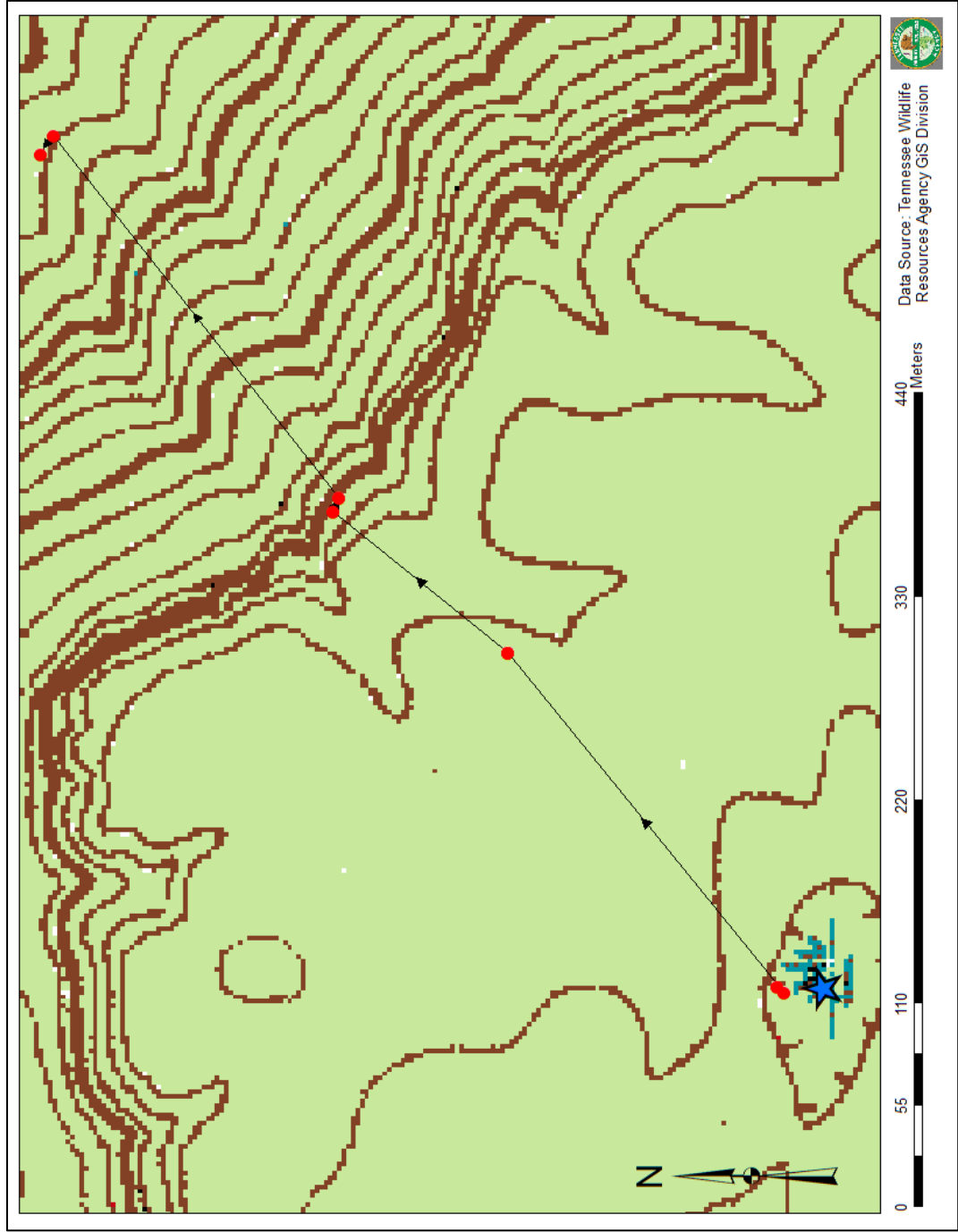
26007 – Lauren. Straight-line distance moved from point of release was 110 m.



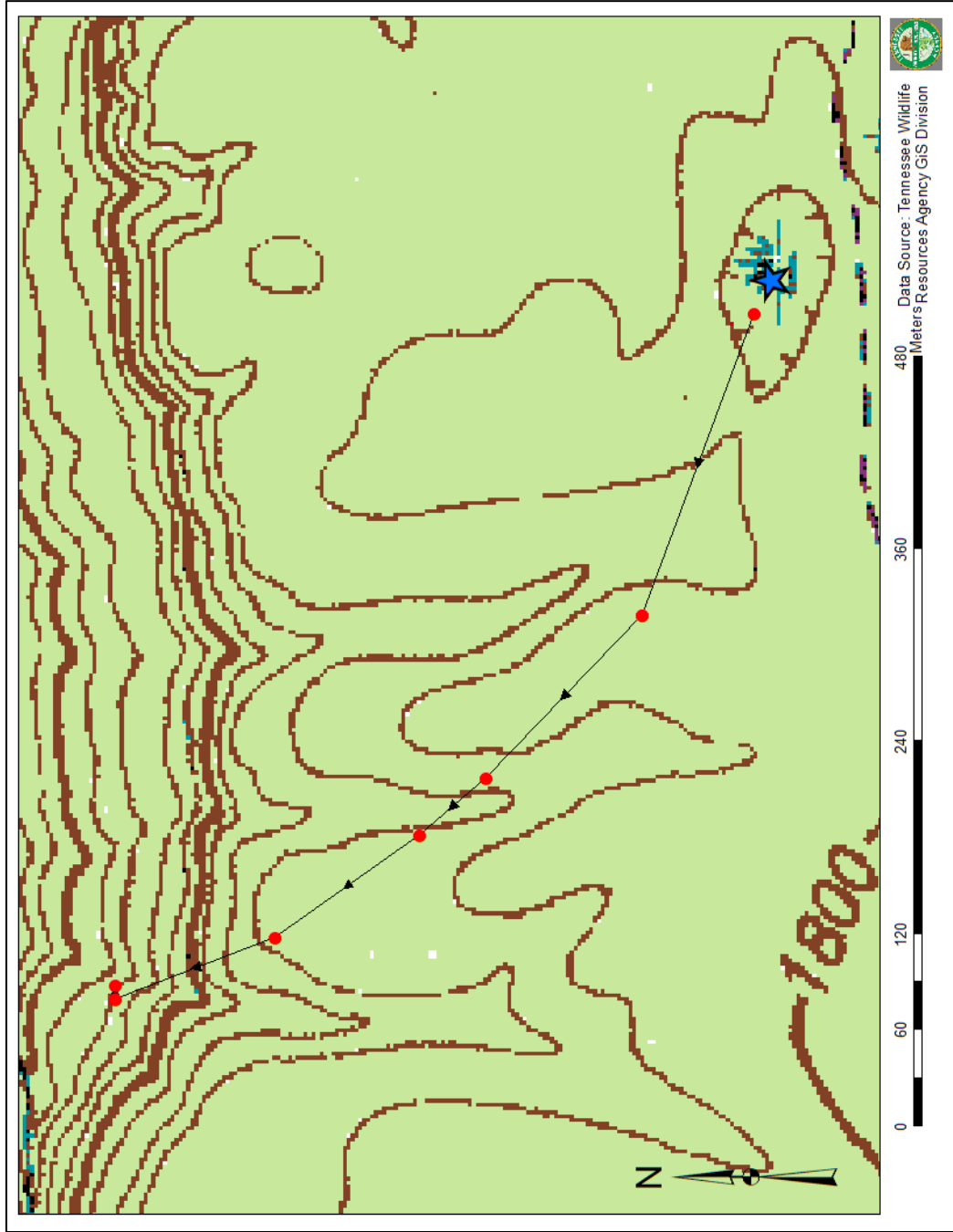
26007 – Elizabeth. Straight-line distance moved from point of release was 220 m.



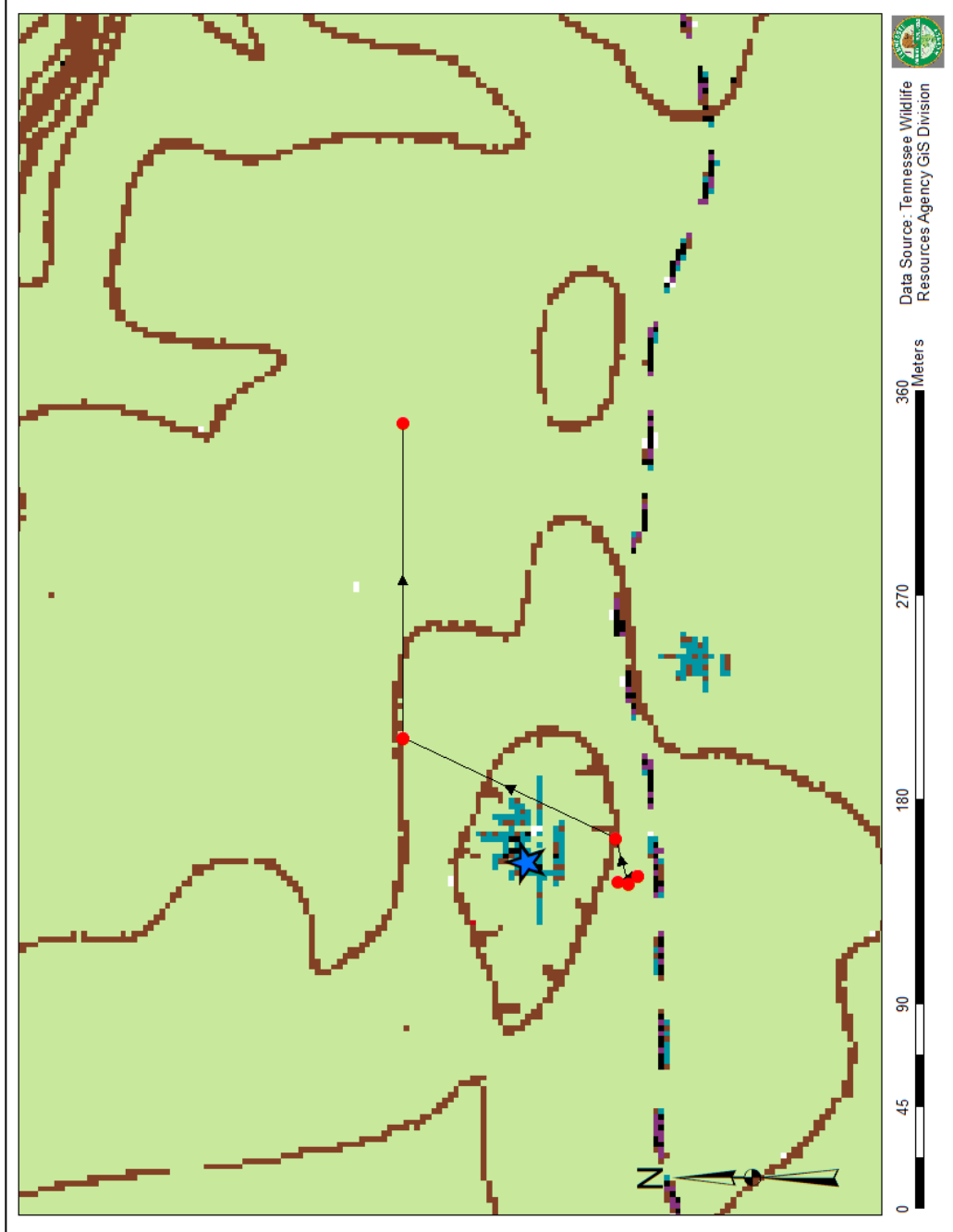
26007 – Macey. Straight-line distance moved from the point of release was 600 m.



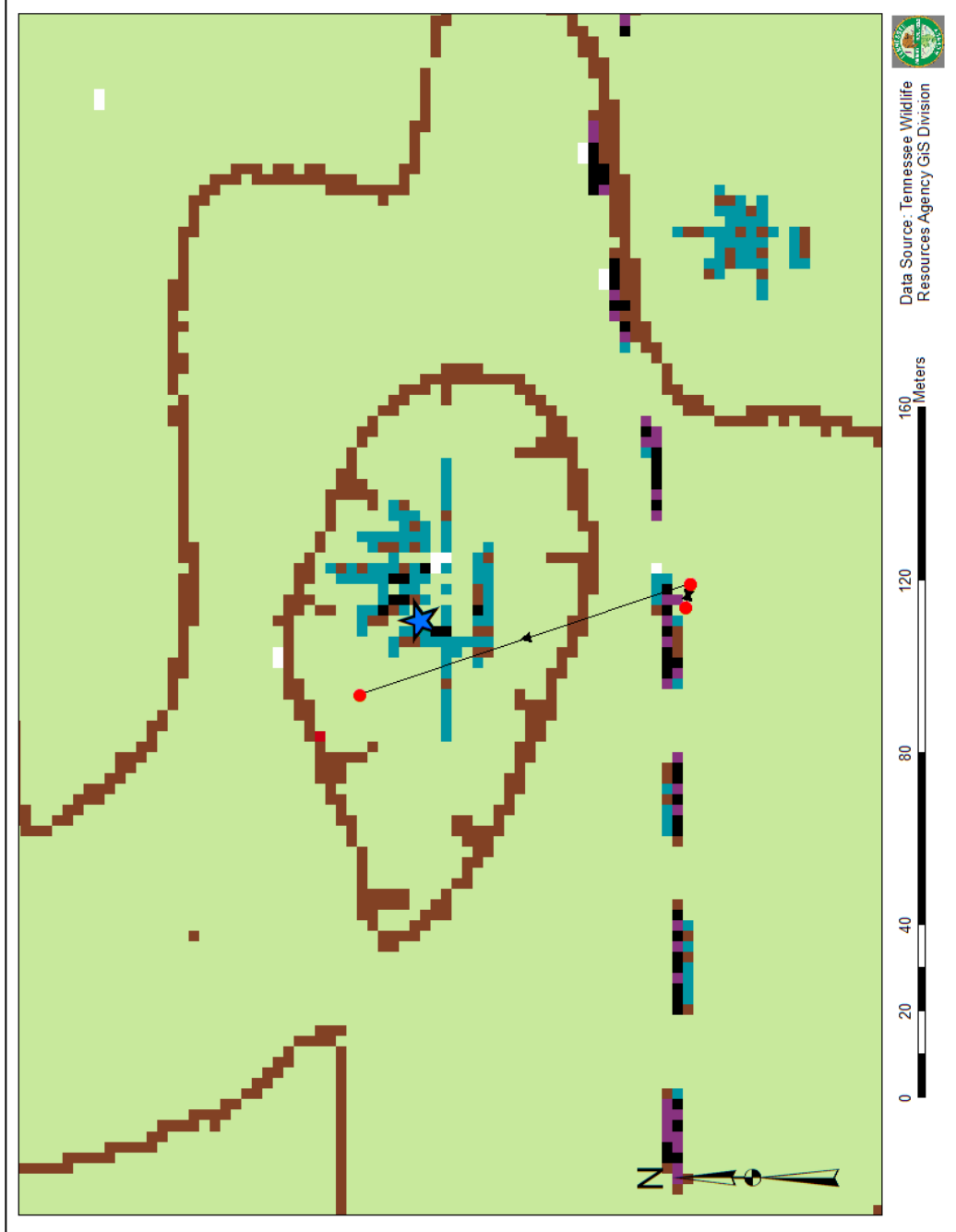
26007 – Carlise. Straight-line distance moved from the point of release was 580 m.



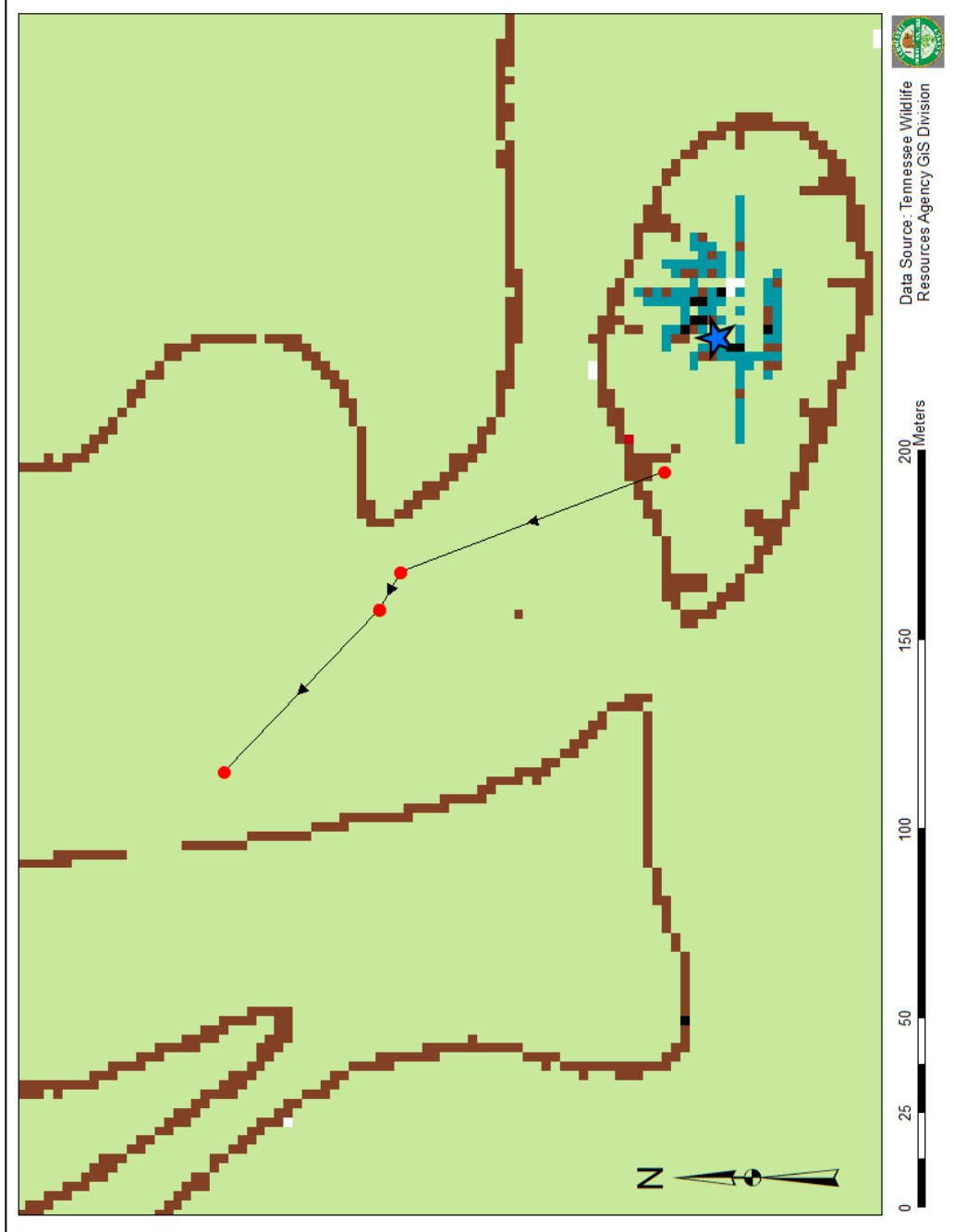
26007 – Sandie. Straight-line distance moved from the point of release was 220 m.



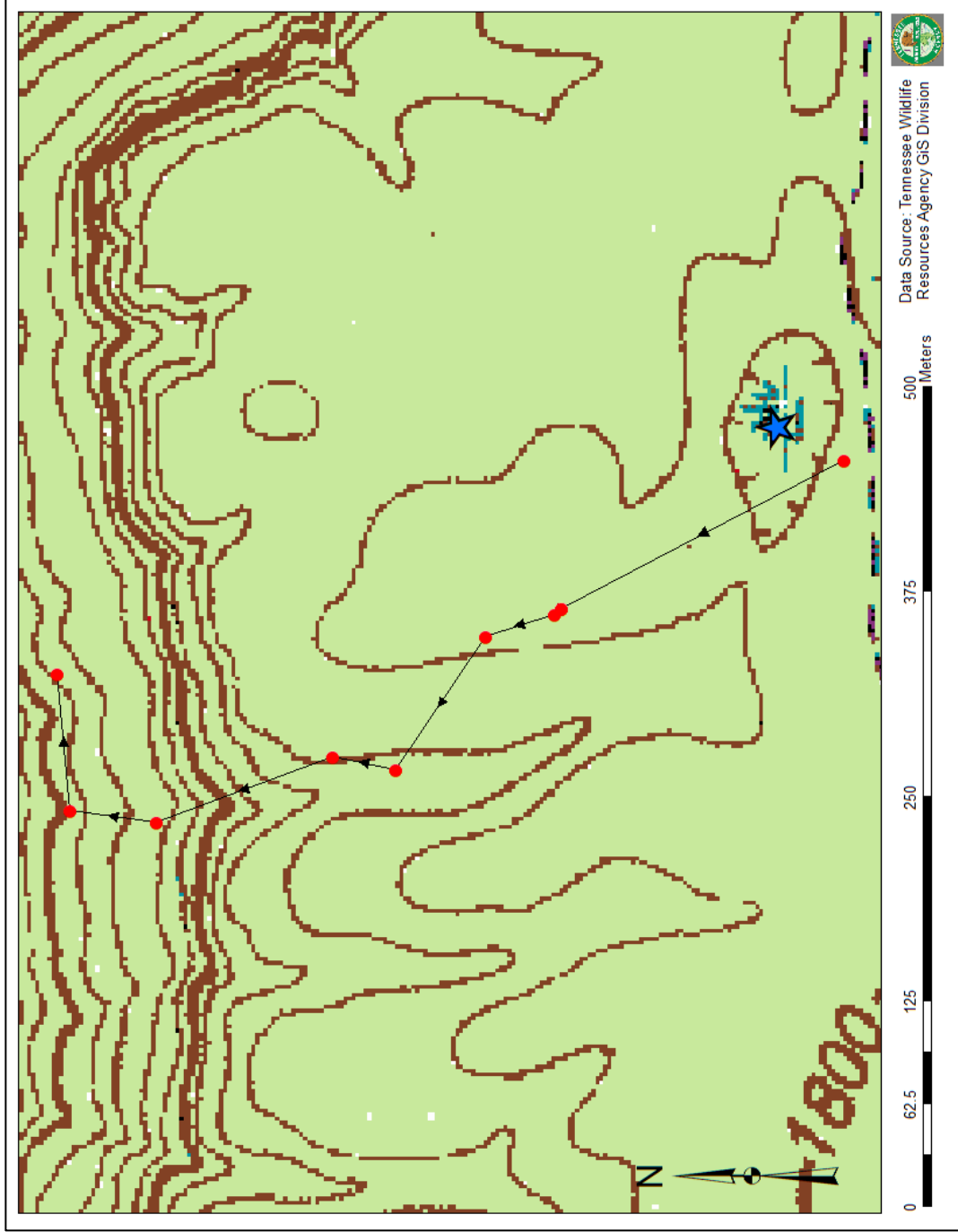
26007 – Emma. Straight-line distance moved from the point of release was 80 m.



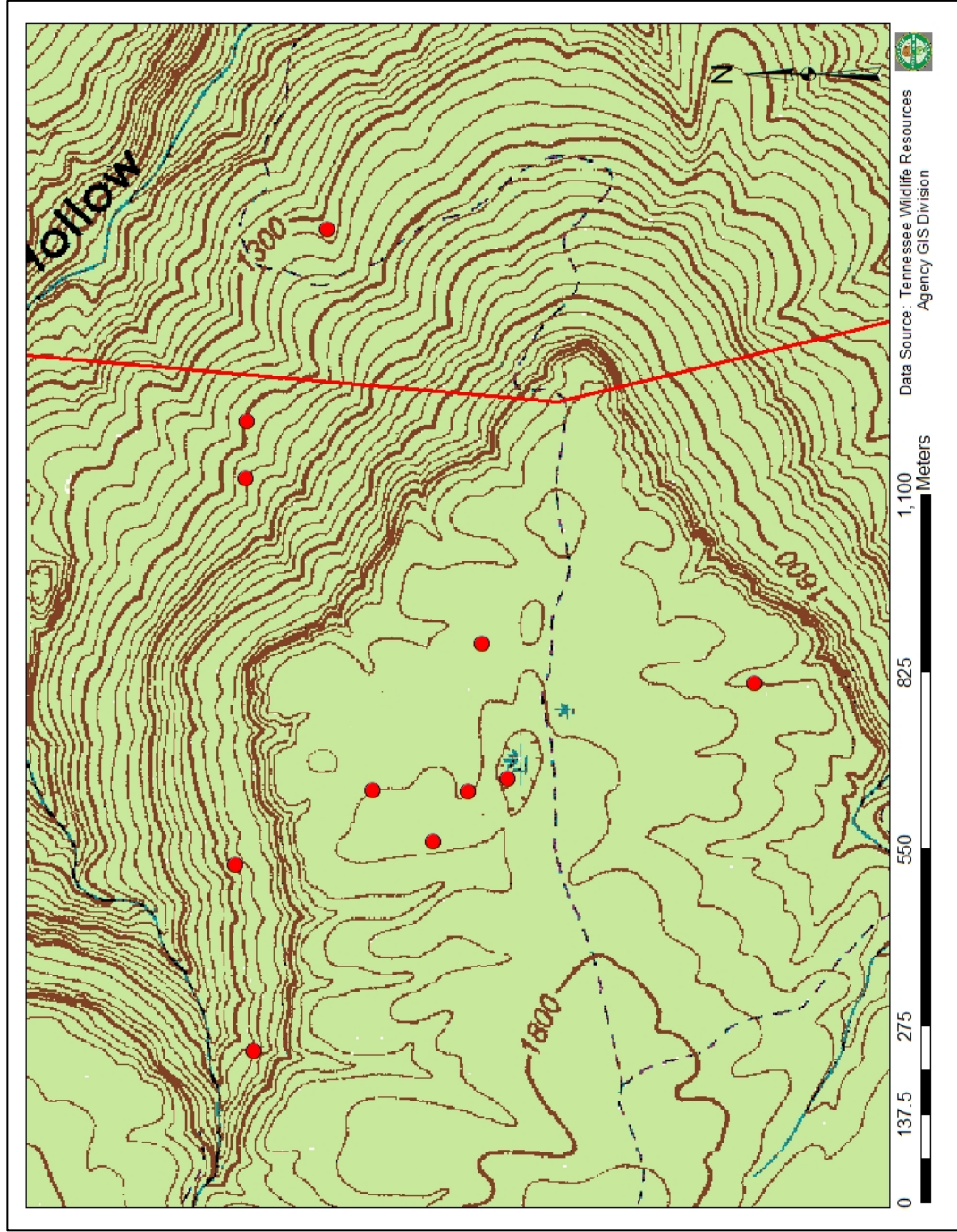
26007 – Hannah. Straight-line distance moved from the point of release was 140 m.



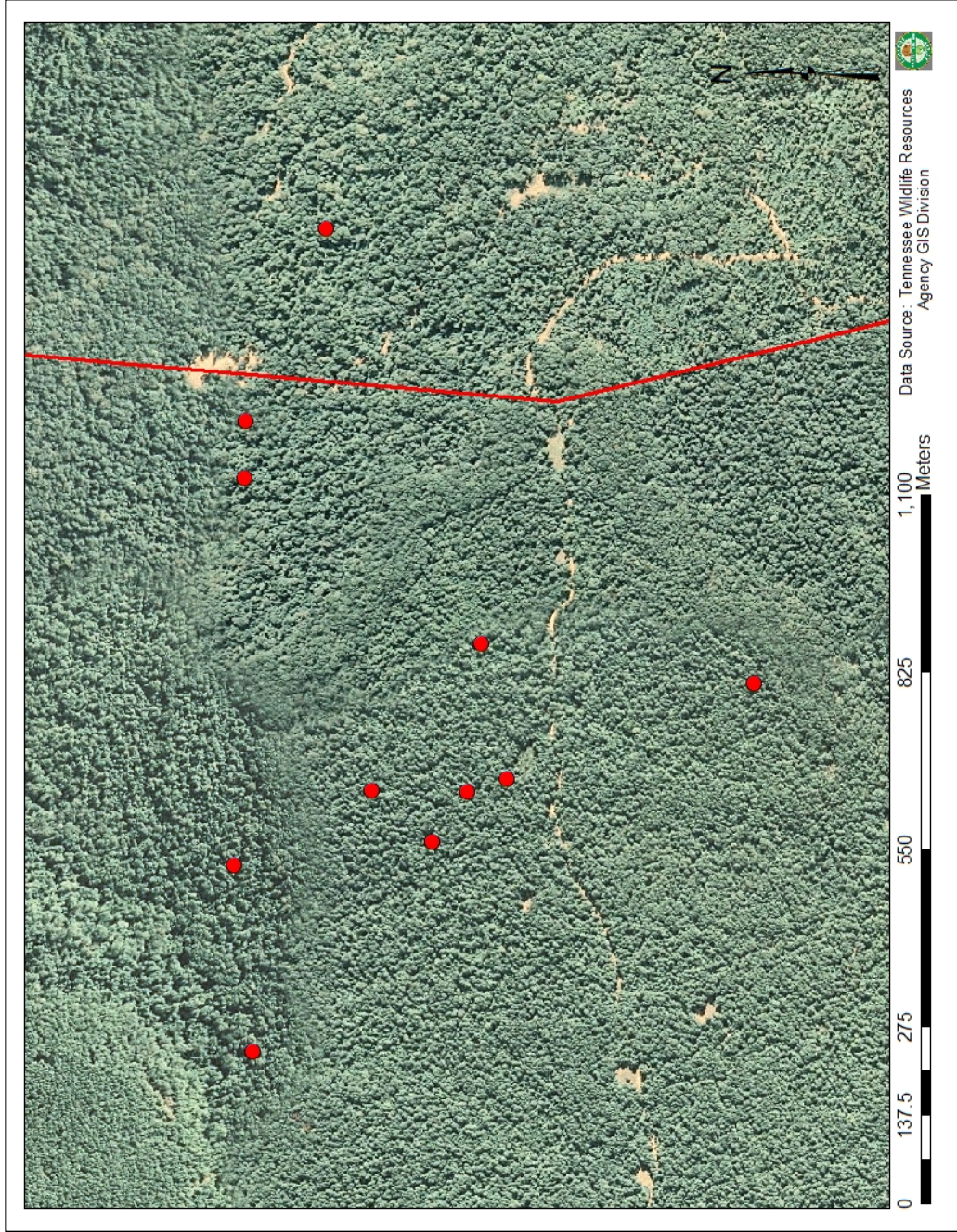
26007 - Zoe. Straight-line distance moved from the point of release was 500 m.



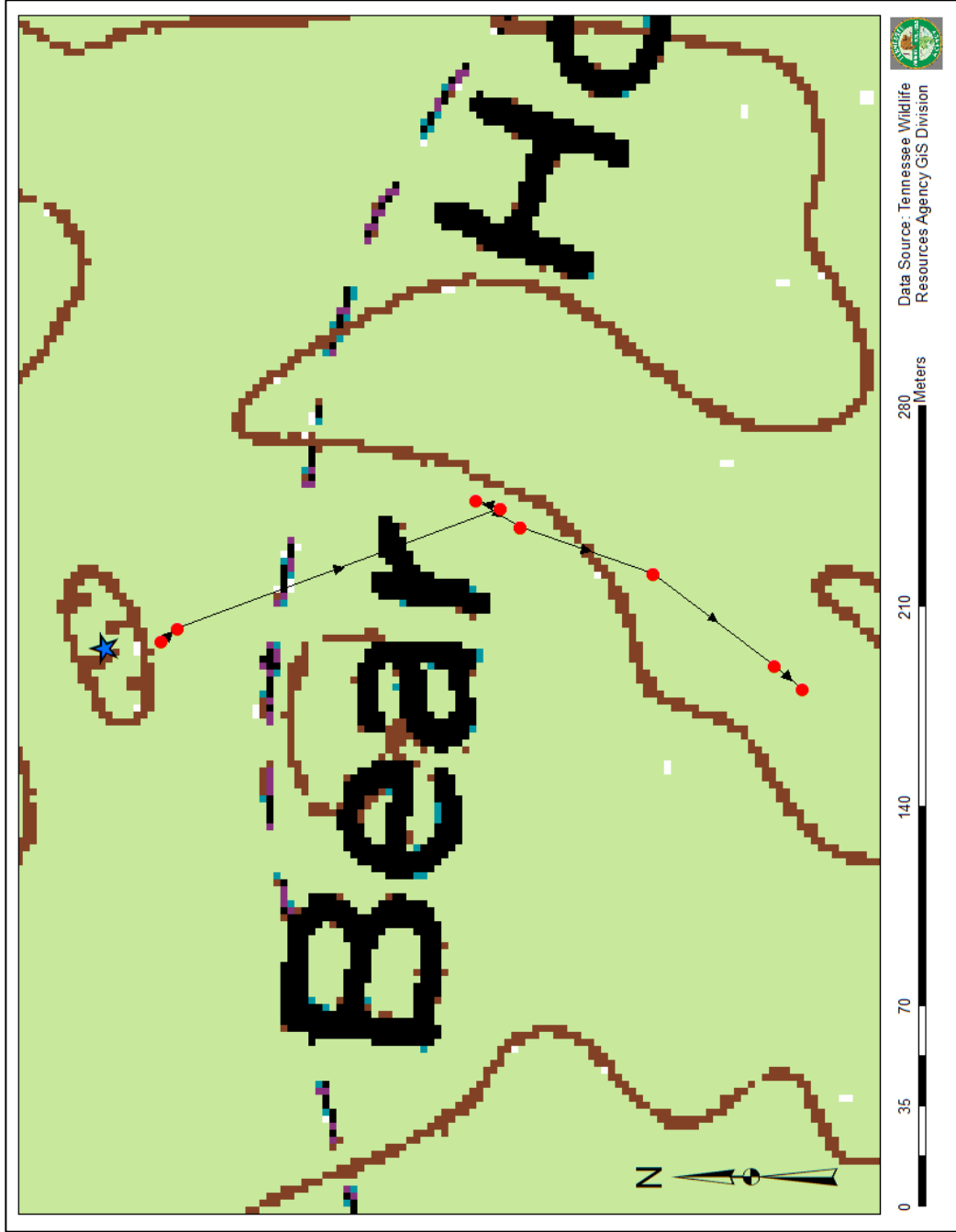
26007 – The last telemetry location recorded for each salamander.



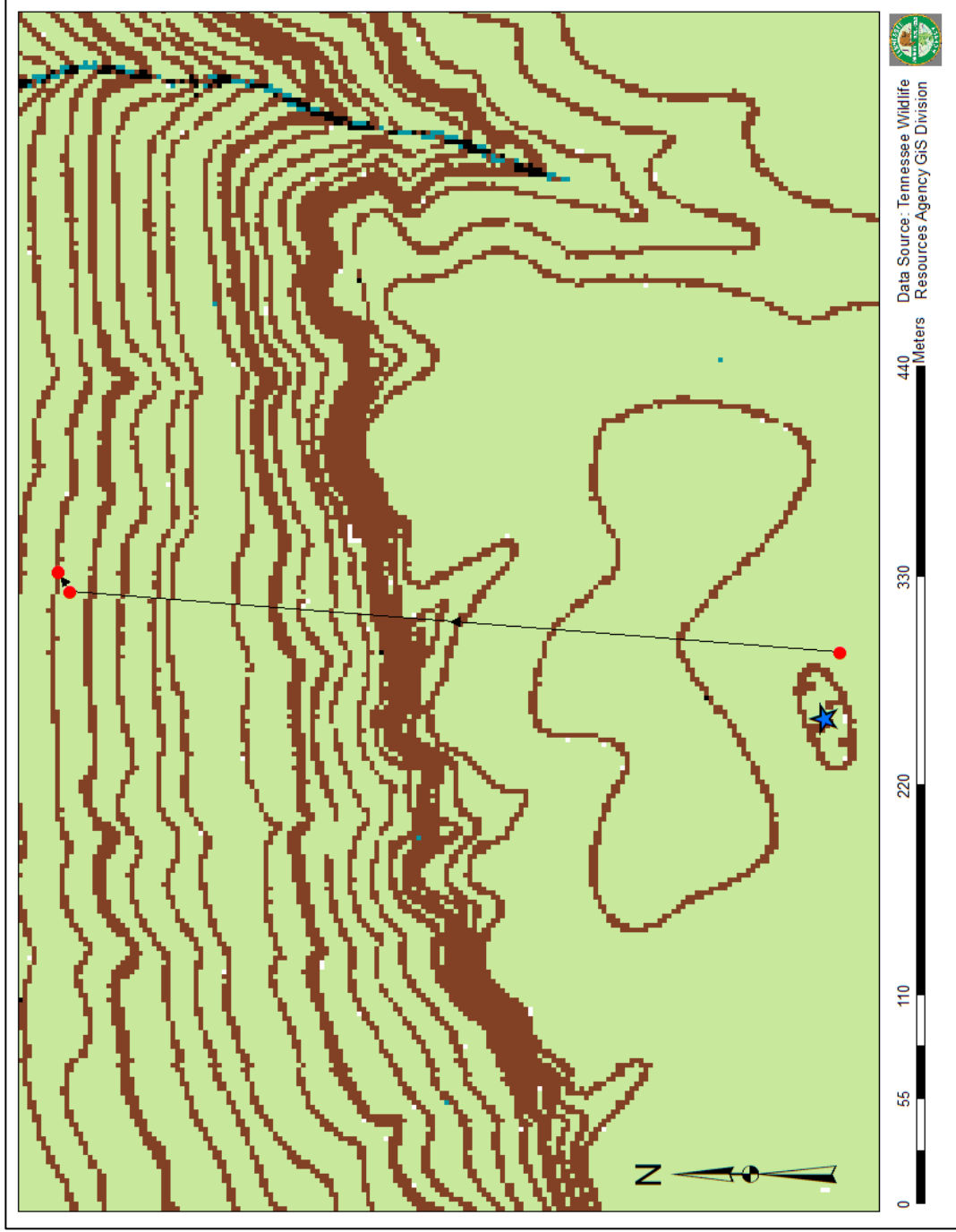
26007 – The last telemetry location recorded for each salamander.



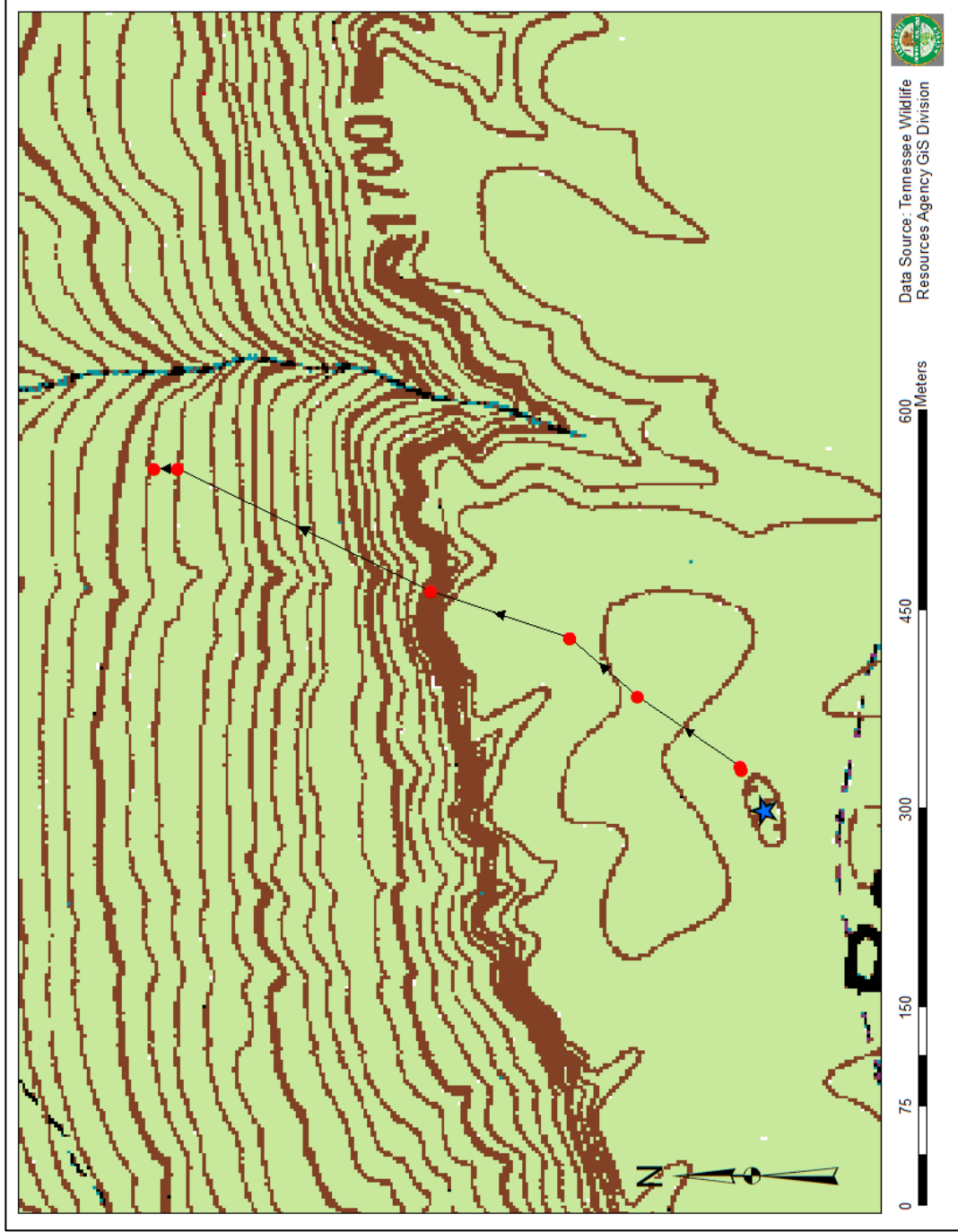
26031 – Megan. Straight-line distance moved from the point of release was 220 m.



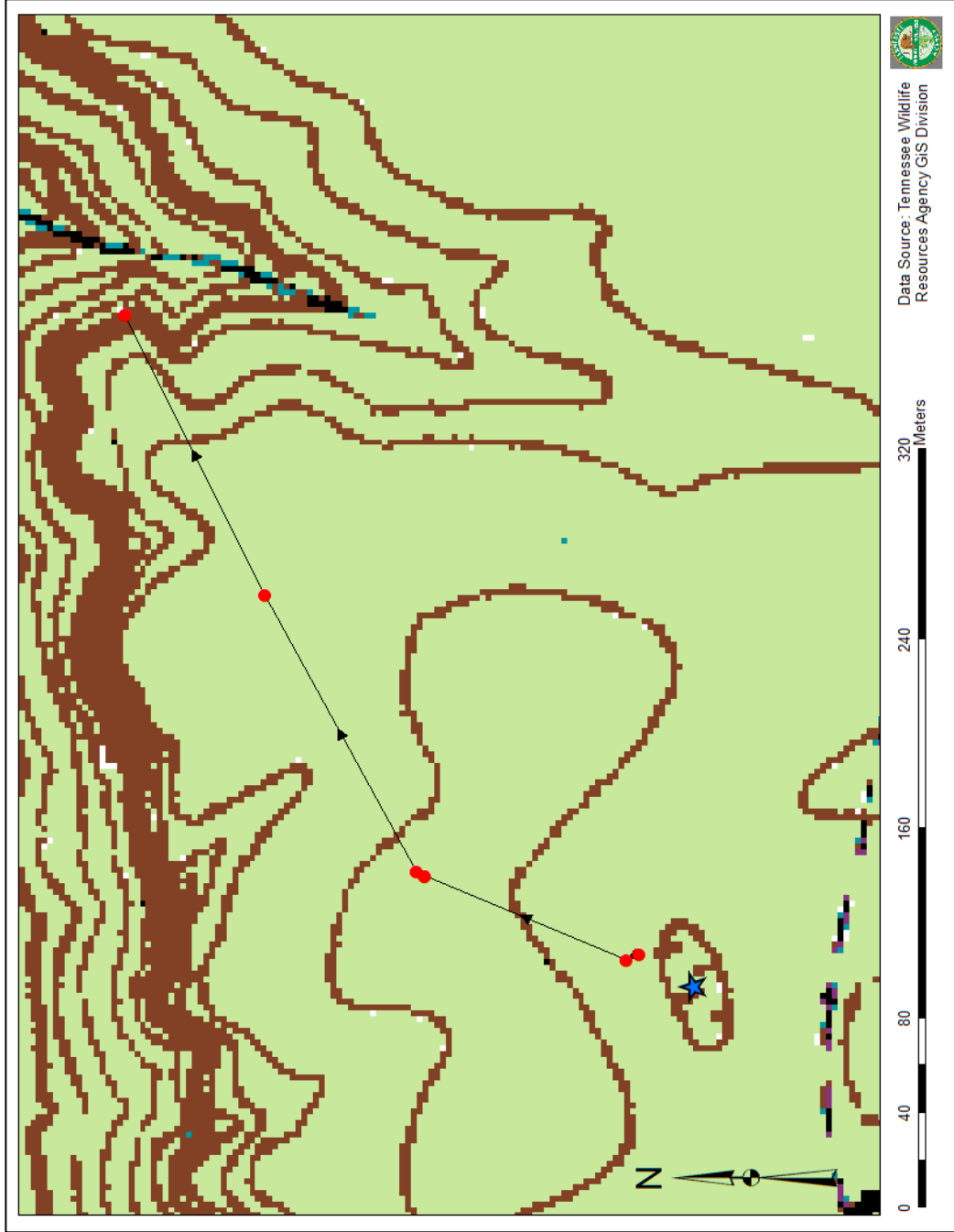
26031 – Lindsey. Straight-line distance moved from the point of release was 410 m.



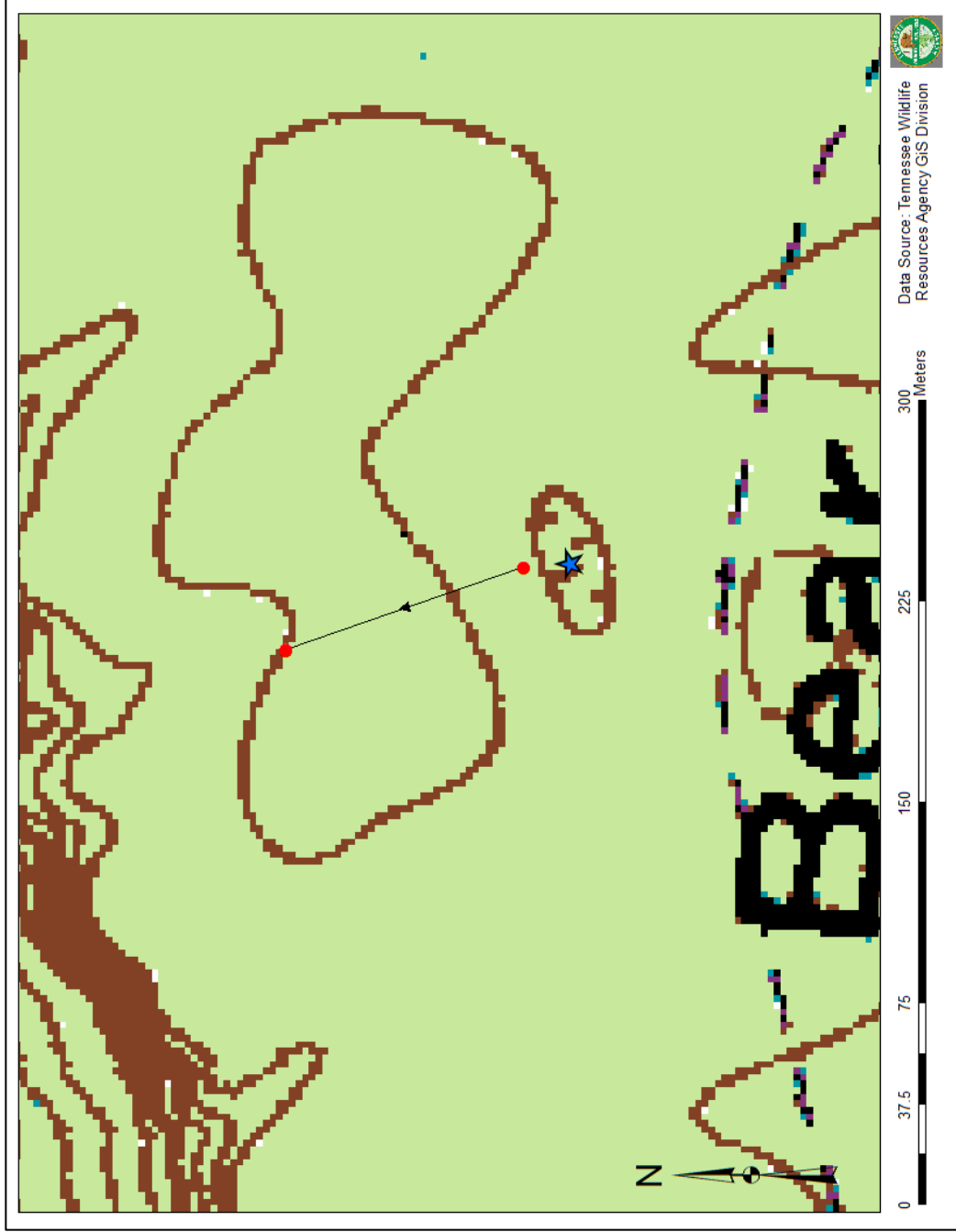
26031 – Ashley. Straight-line distance moved from the point of release was 480 m.



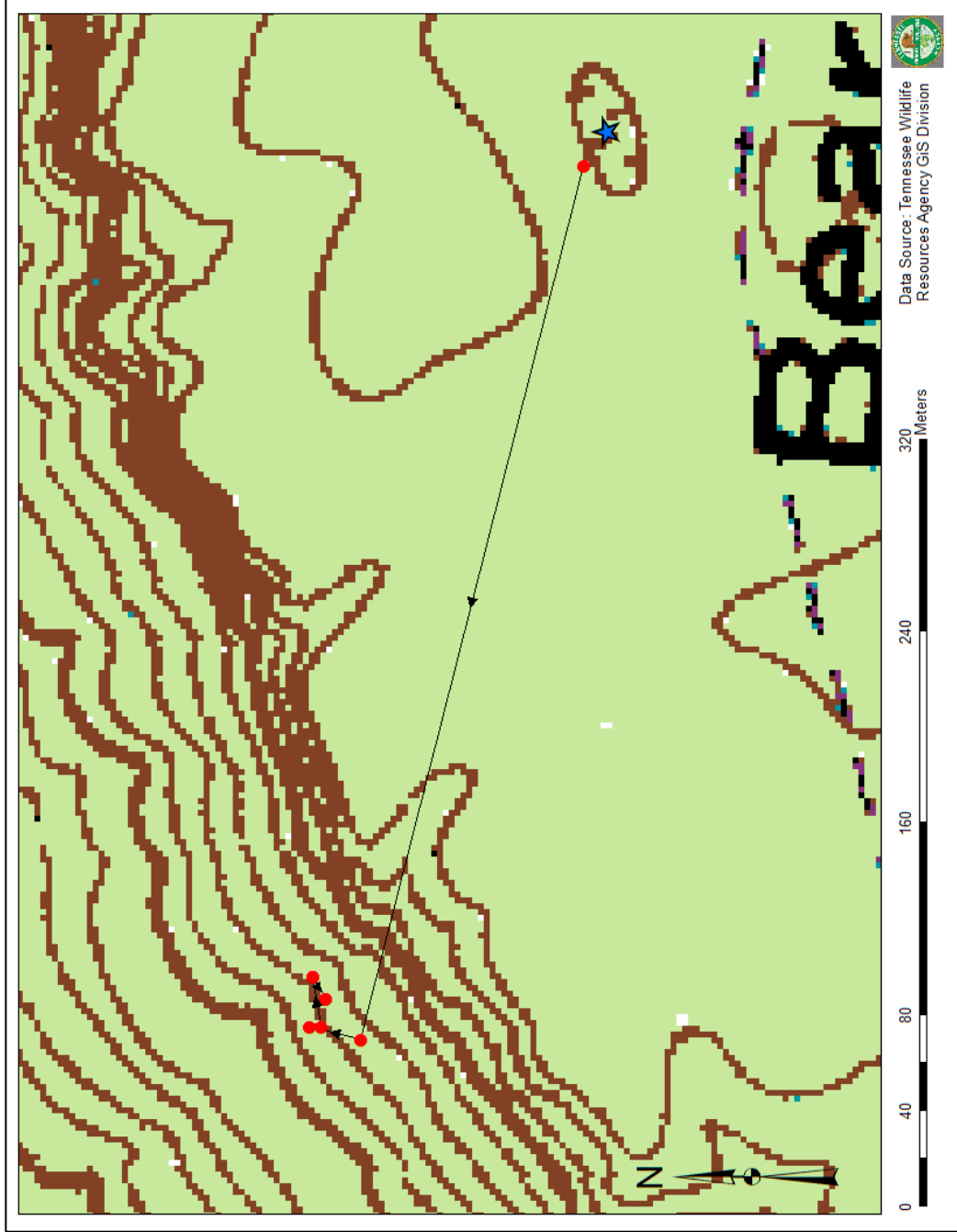
26031 – Daisy. Straight-line distance moved from the point of release was 350 m.



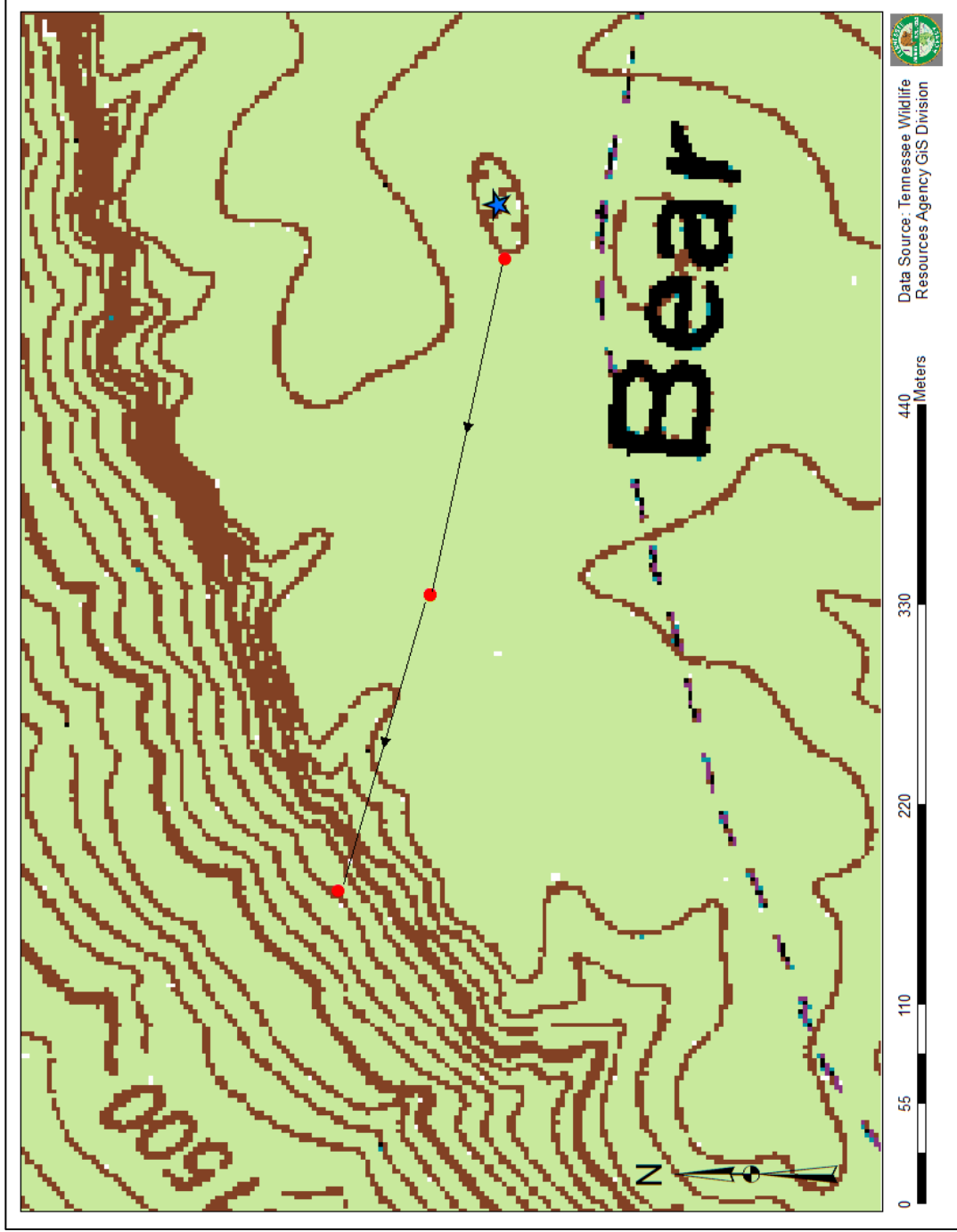
26031 – Olivia. Straight-line distance moved from the point of release was 94 m.



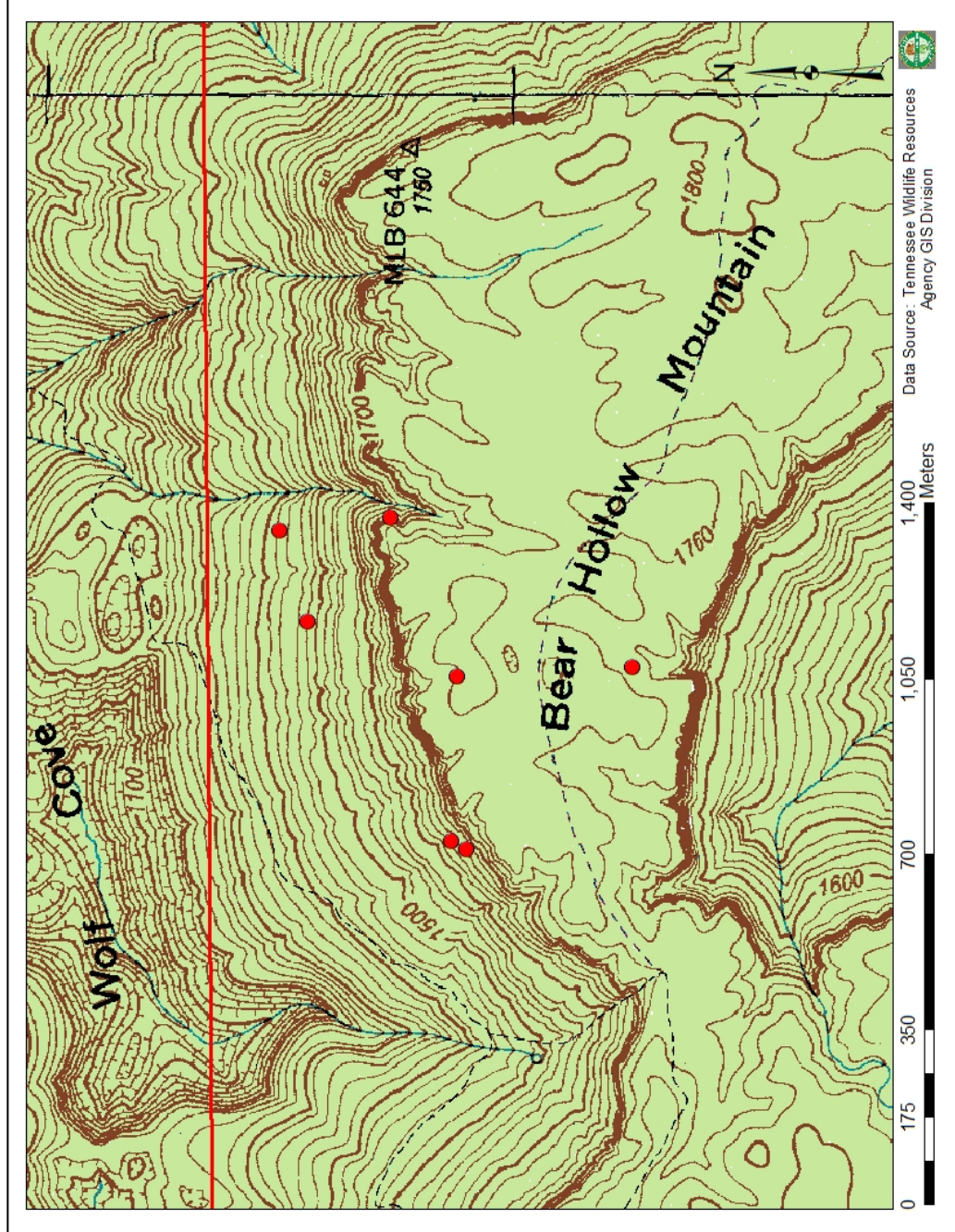
26031 – Kameron. Straight-line distance moved from the point of release was 410 m.



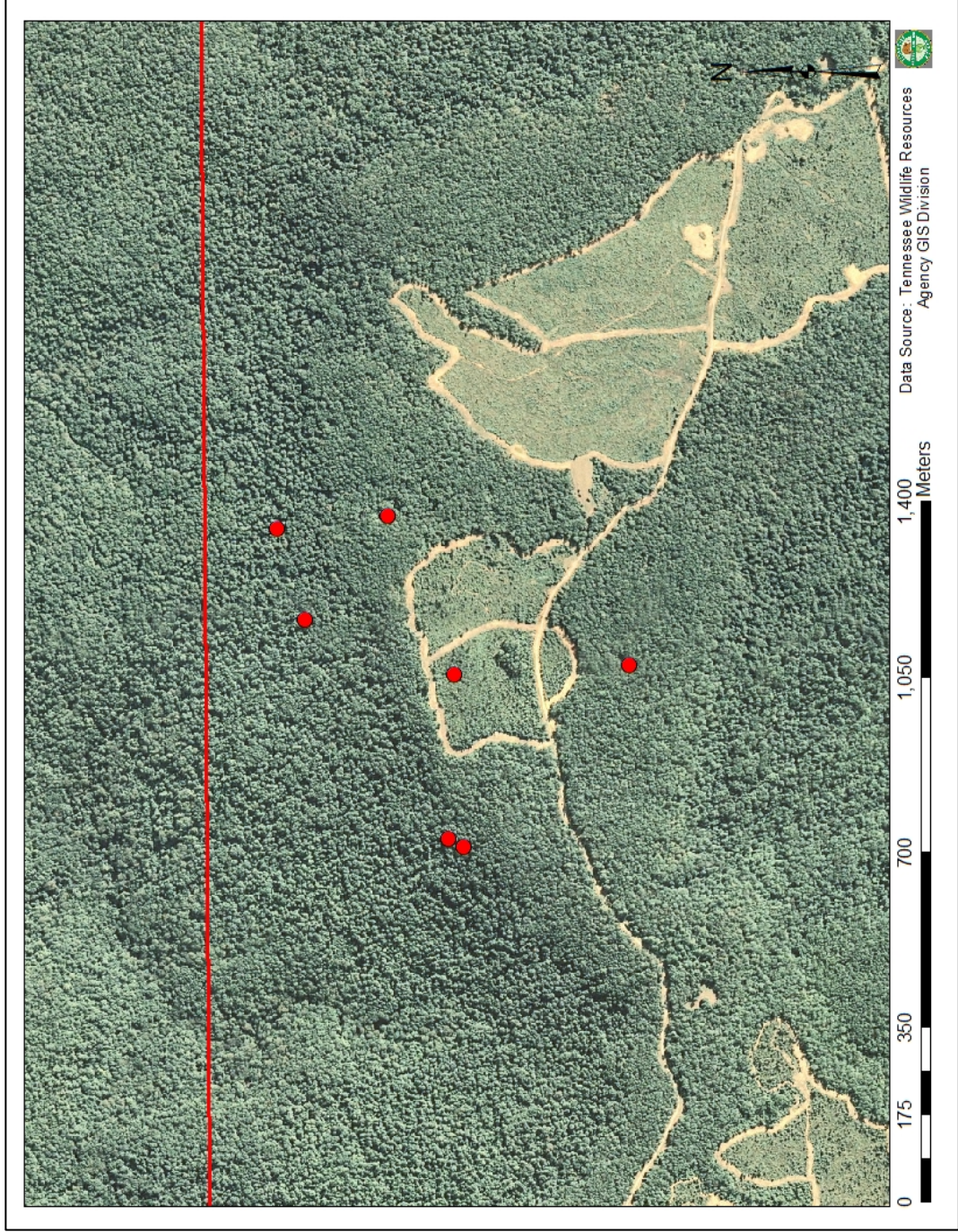
26031 – Jayda. Straight-line distance moved from the point of release was 360 m.



26031 – The last recorded telemetry location recorded for each salamander.



26031 – The last recorded telemetry location recorded for each salamander.



Appendix 5:

IACUC Letters of Approval

October 11, 2010

Investigator(s) Name: Josh Campbell
Investigator(s) Email: jrc6t@mtmail.mtsu.edu

Protocol Title: "Demographics and Phenology of Two Ambystomatid Salamander Populations on the Southern Cumberland Plateau in Franklin County, Tennessee."

Protocol Number: 11-006

Dear Investigator,

The MTSU Institutional Animal Use and Care Committee has reviewed your research proposal identified above and has approved your research under the PHS definition of animal pending the completion of the IACUC training. Approval is granted for three (3) years. **Please note you will need to file a Progress Report annually regarding the status of your study.**

According to MTSU Policy, an investigator is defined as anyone who has contact with animals for research purposes. Anyone meeting this definition needs to be listed on the protocol and needs to provide a certificate of training to the Office of Compliance. If you add investigators to an approved project, please forward an updated list of investigators and their certificates of training to the Office of Compliance before they begin to work on the project.

Any change to the protocol must be submitted to the IACUC before implementing this change. Any unanticipated harms to subjects or adverse events must be reported to the Office of Compliance at (615) 494-8918.

You will need to submit an end-of-project report to the Office of Compliance upon completion of your research. Complete research means that you have finished collecting data and you are ready to submit your thesis and/or publish your findings. Should you not finish your research within the three (3) year period, you must submit a Progress Report and request a continuation prior to the expiration date. Please allow time for review and requested revisions. Your study expires **October 11, 2013**.

Also, all research materials must be retained by the PI or faculty advisor (if the PI is a student) for at least three (3) years after study completion. Should you have any questions or need additional information, please do not hesitate to contact me.

Sincerely,

Emily Born
Compliance Officer