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Population level dynamics of grasshopper sparrow populations breeding on reclaimed mountaintop mines in West Virginia

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POPULATION LEVEL DYNAMICS OF GRASSHOPPER SPARROW POPULATIONS BREEDING ON RECLAIMED MOUNTAINTOP MINES IN WEST VIRGINIA

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ABSTRACT

Population Level Dynamics of Grasshopper Sparrow Populations Breeding on Reclaimed Mountaintop Mines in West Virginia

Frank K. Ammer

During 2001 and 2002, I surveyed three mountaintop mining / valley fill (MTMVF) complexes in southern West Virginia to determine vegetation characteristics important to nest site selection and to estimate nest success for Grasshopper Sparrow populations inhabiting these complexes. I also performed genetic analyses to assess overall population structure, mating system, parentage, kinship, and gender of individuals comprising these populations. A total of 415 grasshopper sparrows were captured and systematic searches of study plots produced 75 active nests. Nest survival for 2001-2002 breeding season (33%) is comparable to survival rates previously reported in the literature. Nest survival rates decreased with increased reclamation age suggesting that vegetation changes and the reduction of bare ground on available grasslands may negatively impact reproductive success. Habitat variables measured at nests and at fixed habitat plots suggest differences in several of the ground cover estimates. Percent green and grass height at 1 m were significantly lower at the nest plots while percent bare ground, percent litter at 1 and 5 m from the nest, grass height at 3 m, shrub stem density, and Robel pole indices at the nest were significantly higher at nest plots. Large reclaimed grassland habitats available on the MTMVF complexes appear sufficient to support breeding populations of grasshopper sparrows; however, habitat will become unsuitable as succession occurs. Genetic analyses suggest low but significant differentiation among mine complexes while the genetic structure of breeding assemblages within mine complexes appears to be homogeneous. The five microsatellite loci screened in this study are robust and appear to be effective in allocating parentage when neither parent is known. Using maximum likelihood methods, I was successful at assigning at least one parent to 80% of the offspring surveyed. The lack of extra-pair paternity within the grasshopper sparrow broods implies a socially and genetically monogamous mating system in this species. Gender assignment data obtained for adult Grasshopper Sparrows by application of the 2550F/2718R primers was in 100% agreement with those collected in the field.

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BACKGROUND ON GRASSLAND BIRDS AND MINING

General Avian Decline-Recent declines in migratory bird populations breeding in the eastern United States have become a major focus of studies addressing avian conservation and biodiversity (Robbins et al. 1989, Askins et al. 1990, Terborgh 1989, Böhning-Gaese et al. 1993). Multi-scale fragmentation, degradation, and alteration of breeding grounds (Robbins et al. 1989, Askins et al. 1990, Herkert 1994), deforestation of tropical wintering grounds (Robbins et al. 1989), and increased nest predation and brood parasitism coupled with increased edge (Johnson and Temple 1990, Hoover and Brittingham 1993) are a few of the factors that have been associated with observed reductions for many songbird species, including species dependent on grassland and early successional habitats (Askins et al. 1990, Smith et al. 1992, Herkert 1994, Warner 1994, Bollinger 1995).

The exact causal mechanisms responsible for recent population declines are still largely unknown due to trend variation across species ranges and because of limited data pertaining to dispersal dynamics within and among specific habitats (James et al. 1992, Donovan and Flather 2002). The reduction and isolation of breeding habitats have been well documented at various spatial scales in forested and urbanized systems (Gutzwiller and Anderson 1987, Robbins et al. 1989, Hill and Hagan 1991, Faaborg et al. 1995, Freemark et al. 1995, Saab 1999, Donovan and Flather 2002), however, studies investigating population declines on grassland and other early successional ecosystems have been limited (Askins et al. 1990, Askins 1993, Herkert 1994).

Grassland and Grassland Bird Declines-The extent and distribution of naturally occurring grassland areas in the eastern United States is largely unknown (Askins 2000). Historically, grassland bird species in the eastern United States persisted in transitory patches of suitable habitat interspersed within the pre-settlement landscape (Askins 1995). Native grasslands were widely distributed and occurred from Maine to Florida and west to coastal areas of Texas (Askins et al. 1990, Vickery 1994). These early successional habitats ranged in size, structure, species composition, and productivity. Substantial evidence suggests that patches of grassland and early successional habitat were frequently created and maintained by natural disturbances such as fire, drought, and wind throw (Askins et al. 1990, Askins et al. 1993, Askins 1996). In addition to natural disturbance, dense beaver populations in the east and grazing by large

herbivores contributed to the pre-agricultural occurrence of early successional vegetation throughout their range.

Accounts from early explorers and colonists suggest that Native American agricultural practices may have had a dramatic effect on the eastern landscape (Askins 1996). Extensive tracts of land were cleared using slash-and-burn methods to create substantial agricultural clearings. These fields were used for crop production until nutrient declines reduced productivity requiring the clearing of additional forested areas. Forest understory also was burned by Native Americans to enhance deer and small game hunting. These practices would have resulted in a pre-settlement landscape comprised of forests and fields in varying successional stages (Askins 1999, Patterson and Sassaman 1988). Subsequent colonization of the eastern region of the United States by early European settlers brought land use practices that drastically altered natural conditions resulting in a landscape mosaic consisting of uneven aged forests, pastures, cropland, and urbanized areas.

While natural grassland systems are limited in West Virginia, grassland areas created by Native Americans for small-scale agriculture and hunting have been documented in the state (Askins 1999). Natural heath barrens, bog communities, grassy balds, and glades occur in the Allegheny Mountain and Ridge and Valley provinces of West Virginia (Strausbaugh and Core 1977). Practically no large natural grassland areas occur in the Allegheny Plateau and Cumberland Plateau regions of West Virginia (DeSelm and Murdock 1993), where this study was conducted. Native grasslands in these physiographic regions are found primarily in upland areas comprised of shallow to moderately deep soil profiles (DeSelm and Murdock 1993). Presently, anthropogenic grassland systems in these regions include airstrips, pastures, cropland, old fields, golf courses, and reclaimed surface mines.

Grassland bird species typically observed using available grassland habitats in West Virginia include Horned Lark (*Eremophila alpestris*) and Dickcissel (*Spiza americana*), that are thought to have moved east from the Midwestern prairies, and species such as the Eastern Meadowlark (*Sturnella magna*), Bobolink (*Dolichonyx oryzivorous*), Savannah Sparrow (*Passerculas sandwichensis*), and Grasshopper Sparrow (*Ammodramus savannarum*), that are assumed to have expanded into these areas from eastern coastal and marsh grasslands (DeSelm and Murdock 1993, Askins 1999). Other grassland species that have been documented in the state include Henslow's sparrow (*Ammodramus henslowii*), vesper sparrow (*Pooecetes*

gramineus), and field sparrow (*Spizella pusilla*) (Buckelew and Hall 1994, Whitmore and Hall 1978, Whitmore 1978).

Mining-Historically, coal mining in West Virginia has been an important economic and cultural industry. John Peter Salley first discovered coal in the southern region of the state in 1742. Extensive coal reserves were known to occur throughout much of West Virginia; however, large-scale extraction did not begin until the mid-1800s. Prior to the increase of large-scale industry in the east, there was little incentive to exploit coal as a marketable resource.

The two main coal extraction methods that have been used in West Virginia are underground and surface mining. Underground extraction methods include drift, slope, and shaft mining. Drift mines (highwall mines) bore into the side of a hill to extract coal reserves within the hill. Slope mines consist of sloping tunnels that normally originate in valley bottoms and are built to various depths to extract specific coal seams. Shaft mines are the deepest mines consisting of a vertical shaft that extends from the surface to the desired coal seam (National Mining Association 1996).

Surface-mining methods include area, contour, augur, and mountaintop mining/valley fill extraction. Area mines are used to extract shallow coal reserves over an expansive area where the terrain is relatively level. Contour mining methods are designed to extract coal in steep, hilly, or mountainous terrain. With this method, a portion of the overburden is extracted along the coal outcrop on the face of a hill creating a bench at the level of the seam. After extraction, the resulting overburden is replaced and compacted to return the landscape to its approximate original contour. Augur mines use mine benches to extract seams that cannot be extracted by contour mining (National Mining Association 1996).

Mountaintop mining / valley fill (MTMVF) complexes are large-scale area mines that target coal seams that cannot be economically mined with other conventional techniques. MTMVF techniques were first used in West Virginia in 1967 on the Cannelton Mine located approximately 50 km southeast of Charleston. This technique involves the removal of large quantities of overburden from mountaintops to expose multiple coal seams in the Kanawha and Allegheny formations (Nieman and Merkin 1995, Fedorko and Blake 1998). After coal extraction, resulting overburden is placed back onto the flattened ridgeline and in adjacent stream valleys that are commonly referred to as valley fills or head-of-hollow fills. Valley fills are

terraced and compacted to increase slope stability and also fitted with a rock drainage channels to minimize erosion (Greene and Raney 1979, Robins and Hutchins 1984). The 1977 Surface Mining Control and Reclamation Act (SMCRA) requires that the overburden removed in the extraction process be used to re-grade slopes into the approximate original contour (AOC) of the mined area (OSM 1999). Many past MTMVF permits were granted with an AOC variance that allows the formation of a plateau or gently rolling topography containing no highwalls.

Reclaimed areas are replanted with a diverse mixture of native and exotic grass and forb species compliant with the 1977 Surface Mining Control and Reclamation Act. Commonly used species in West Virginia include tall fescue (*Festuca arundinacea*), broomsedge (*Andropogon virginianus*), orchard grass (*Dactylis glomerata*), and perennial ryegrass (*Lolium perenne*), Birdsfoot-trefoil (*Lotus corniculatus*), alfalfa (*Medicago sativa*), sericea (*Lespedesa cuneata*), sweet clover (*Melilotus alba*), and mammoth red clover (*Trifolium pratense*) (personal communication John McDaniel, Arch Coal 2001). Early successional communities on these complexes are temporary and will eventually revert to forested habitat. Successional changes on MTMVF grasslands proceed at a slower rate than on non-mined areas due to the poor quality, rocky soils present after reclamation (Bajema et al. 2001, DeVault et al.2002).

Grassland bird use of reclaimed mines-Studies investigating the effects of surface mining on bird population dynamics have increased over the past few decades to examine how grassland and early successional species respond to these novel habitats. Most early studies were conducted on small surface mined lands that were either actively reclaimed or naturally revegetated through succession (Yahner and Howell 1975, Chapman 1977, Crawford et al. 1978*a b*, Whitmore 1978, Whitmore and Hall 1978, Wray et al. 1978, Allaire 1979, Whitmore 1979, Wray 1979, Wackenhut 1980, Whitmore 1980, Whitmore 1981, Strait 1981, LeClerc 1982, Wray 1982, Wray et al 1982). Most studies described the type of mining activity used for mineral extraction and may be characterized accordingly. Studies also may be characterized by the questions asked and overall study design. The three most common types of studies in the literature examined bird use of mined habitats, bird-habitat relationships, and reproductive success on mine sites (Allaire 1980).

The majority of studies investigating avian use of small-scale surface mines suggest that birds follow a pattern of habitat use that is typical of what is seen in natural systems (Allaire 1980). Common bird communities of recently reclaimed areas are composed mainly of

grassland bird species. Grasshopper Sparrows, Eastern Meadowlarks, Savannah Sparrows, Vesper Sparrows, Horned Larks, and Red-winged Blackbirds commonly dominate these communities (Whitmore and Hall 1978, Wood et al. 2000). In addition, several authors have noted that the presence of reclaimed mines in eastern states have allowed the range expansion of several grassland species, including Savannah Sparrows, Dickcissels, Bobolinks, and Short-eared Owls (*Asio flammeus*) (Chapman 1977, Whitmore 1978, Whitmore and Hall 1978, Allaire 1979, LeClerc 1982, Wray 1982, Ammer and Wood 2002).

Unmanaged grassland habitats on mined lands are temporary and will become unsuitable for grassland communities as succession occurs on these sites. Successional changes will ultimately cause a community level shift in songbird species using these areas. Brewer (1958) surveyed bird populations on a naturally revegetated mine in Illinois and described 44 species using the mine site. The majority of species encountered were forest-edge species, however, he noted that species composition shifted over time as succession proceeded towards hardwood forest. Karr (1968) also suggested that species composition and richness shifted as succession proceeded on surface mine sites in Illinois.

Several studies have documented the relationship between bird abundance, nest success, and habitat variables on reclaimed surface mines (Chapman 1977, Chapman et al. 1978, Whitmore 1979, Wray 1979, Wackenhut 1980, Strait 1981, LeClerc 1982). All of these studies except Chapman (1977) and Chapman et al. (1978) were conducted on relatively small (<45 ha) reclaimed mine sites in West Virginia and offer limited insight into population dynamics and overall success of grassland species breeding on large surface mines.

Chapman (1977) and Chapman et al. (1978) examined relationships between bird abundance and vegetation characteristics on contour mines in southwest Virginia ranging in age from 11-27 years. Territory mapping was used to census birds on 12 study areas that varied in degree of revegetation. Results from their study suggested a positive correlation between percent ground cover and the number of species using these habitats and that vertical structure of vegetation was an important predictor of species richness. The West Virginia studies were conducted on five reclaimed surface mines ranging in age from three to six years and in size from 9.1-ha to 41.5-ha. These studies examined reclamation strategies to promote colonization, habitat selection, and the effect of vegetative structure on the reproductive success of grassland birds. Whitmore (1979) examined the effects of vegetation structural change on Grasshopper

Sparrow density. His data suggested that changes in species density were related to the overall density of preferred ground cover. His data also suggest that Grasshopper Sparrows select breeding territories containing a high percentage of bareground (24%) for foraging and courtship activities. Grasshopper Sparrow densities were high in areas that provided adequate bareground cover, but decreased substantially as the amount of litter cover increased. These trends were similar for Savannah Sparrows and Vesper Sparrows, whereas Eastern Meadowlarks preferred areas with more dense vegetation. Whitmore (1979) suggested that the density of ground cover was the key variable affecting habitat selection in several grassland bird species.

Wackenhut (1980) examined habitat selection by Horned Larks on several reclaimed sites. These data indicated that this species prefers territories containing a high degree of bareground with little forb, grass, and shrub cover. This study also proposed that vegetative structure has little influence on nesting success in Horned Larks. Wray (1979) and Strait (1981) examined habitat selection and niche separation of three grassland sparrow species (Vesper, Grasshopper, and Savannah). Wray (1979) suggested that preferred vegetative structure near nest sites differed among the sparrow species and that successful nests had greater vegetation biomass and height than unsuccessful nests. Results from Strait (1981) implied that Vesper Sparrows prefer more open areas than the other two species and that vegetation surrounding Vesper Sparrow nests did not appear to affect the probability of nest predation. Overall, these studies concluded that habitat preferences are species-specific and that vegetative characteristics, structure, and composition influence nest site selection and nest success.

LeClerc (1982) examined the relationship between vegetative structure and bird species on 23 surface mines in northern West Virginia. Her data suggest that percent grass cover, percent bare ground, litter depth, and effective height of vegetation can be used to discriminate among mine sites. These data also suggest that bird communities differ significantly between contour mines and surface mines. This study also addressed habitat relationships among mine sites for six grassland bird species. Savannah and Grasshopper Sparrows preferred areas with a high degree of forb and bare ground cover but minimal shrub cover. Eastern Meadowlarks preferred mined areas with low shrub cover and vertical density but greater grass cover. Vesper Sparrows preferred mines with less grass cover, a deep litter layer, and a high forb and shrub cover layer. Horned Larks were associated with mines containing low grass and shrub cover, whereas Red-winged Blackbirds preferred mines with high grass and forb cover.

Several studies have documented the nesting success of grassland birds breeding on reclaimed mines in Preston County, West Virginia (Wray et al. 1978, Wray 1979, Wackenhut 1980, Strait 1981, Wray 1982, Wray et al. 1982). All of these studies concentrated on grassland sparrows with the exception of Wackenhut (1980) who focused on Horned Larks. Data from these studies suggest that grassland species breeding in this region may be double-brooded or triple-brooded, and that predation accounted for 48% of nest failure. The mean clutch size of Vesper Sparrows, Grasshopper Sparrows, Savannah Sparrows, and Horned Larks ranged from 3.20-5.25 eggs per brood. The probability of an egg surviving to fledgling stage ranged from 0.05-0.32, while the number of fledglings produced per hectare ranged from 0.05 to 1.45. Average clutch sizes from these studies are similar to those published in the literature for these species; however, the number of fledglings produced per hectare was lower than normally expected in natural grasslands (Wray et al. 1982). Fledging success ranged from 4.3-6.9% for Grasshopper Sparrows, from 3.6-4.8% for Vesper Sparrows, from 5.4-6.4%, for Savannah Sparrows, and was 6.6% for Field Sparrows (Strait 1981). Wray et al. (1982) suggested that reclaimed surface mines might not provide suitable nesting habitat for nesting sparrow species due to poor breeding success on these sites. Wackenhut (1980) examined 47 active Horned Lark nests on surface mines and found that nest success estimates for this species were low (4.8%) with 75% of the losses attributed to depredation.

Data concerning species richness, nest site selection, and nesting success of birds using large MTMVF grasslands in the east are scarce. Several recently completed studies in southern Indiana (Bajema and Lima 2001, DeVault et al. 2002) examined breeding bird communities using large reclaimed surface mine sites (110-3200 ha); however, topography, climate, vegetation composition, and overall landscape structure in Indiana differs considerably from that in West Virginia. These differences make assumptions about nest survival of songbirds breeding on reclaimed mine sites in West Virginia difficult, mainly because it is unfeasible to predict which if any of the variables identified by these other studies are limiting factors to breeding success.

In a study of three MTMVF complexes in southwestern West Virginia during 1999 and 2000, Wood et al. (2001) found that Grasshopper Sparrows were the most common grassland bird species observed. Nest densities, however, were generally low, particularly given the high abundance of singing males on the study areas. It may be that males are attracted to reclaimed

areas but are not able to establish territories or to find mates. This needs to be investigated further before decisions are made regarding the importance of these newly created grassland habitats to bird populations.

GRASSHOPPER SPARROW LIFE HISTORY

Taxonomy and Geographic Distribution-The Grasshopper Sparrow was first described and named *Fringilla savannarum* in 1789 by Johann Gmelin using specimens from Jamaica (Rising 1996). Louis Pierre Viellot first described the species in North America in 1818 using specimens from New York (Rising 1996).

Grasshopper Sparrow breeding range encompasses much of North America south from British Columbia, east to Quebec and New Brunswick, south to central Georgia and Alabama, Tennessee, Arkansas, and Texas, southeast Colorado, northeast New Mexico, Wyoming, Montana, Idaho, Utah, Washington, Oregon, and California (Vickery 1996, Rising 1996, Beadle and Rising 2002). Resident populations occur in Arizona, Florida, Mexico, Guatemala, Belize, Honduras, Nicaragua, Costa Rica, Panama, Jamaica, Puerto Rico, Columbia, Ecuador, and the Netherlands Antilles (Vickery 1996, Rising 1996, Beadle and Rising 2002). This species also has a large winter range that extends south from North Carolina, Tennessee, Arkansas, Oklahoma, Arizona, and California, south through Baja California, and Mexico and Central America (Ehrlich et al 1988,Vickery 1996, Rising 1996, Beadle and Rising 2002).

A total of twelve sub-species have been described (Paynter and Storer 1970, Olson 1980), however evidence of differentiation is weak for all sub-species (Vickery 1996). The four Caribbean races of Grasshopper Sparrows *Ammodramus savannarum savannarum*, *Ammodramus savannarum borinquensis*, *Ammodramus savannarum intricatus*, and *Ammodramus savannarum caribaeus* are residents of Jamaica, Puerto Rico and various Caribbean island systems. The four Central American races of Grasshopper Sparrows *Ammodramus savannarum cracens, Ammodramus savannarum bimaculatus, Ammodramus savannarum beatriceae*, and *Ammodramus savannarum caucae* are resident and occur from southern Mexico to northern Ecuador. The four remaining sub-species breed in North America. The Florida Grasshopper Sparrow (*Ammodramus savannarum floridanus*) is a year round resident of central Florida. The eastern Grasshopper Sparrow (*Ammodramus savannarum*

pratensis) has a breeding range that extends from the eastern costal region west to Wisconsin and Oklahoma. The western Grasshopper Sparrow (*Ammodramus savannarum perpallidus*) breeds in western North America south to Arizona. The Arizona Grasshopper Sparrow (*Ammodramus savannarum ammolegus*) breeds in southeastern Arizona and northern Sonora (Vickery 1996). *Breeding and Nesting Habitat*-Grasshopper Sparrows are typically associated with moderately open grassland and prairie systems with bareground patches (Whitmore 1979, Vickery 1996) during the breeding season. Habitat selection patterns differ across the range of this species, depending on the vegetative composition of the grassland ecosystem. Grasshopper Sparrows are proposed to be area sensitive and are more likely to occupy larger tracts of land (Samson 1980, Vickery et al. 1994). This species generally avoids grassland areas with extensive tree and shrub cover (Vickery 1996).

Grasshopper Sparrow nests are normally cup shaped, lined with grass or hair, and built into a clump of grasses and forbs by the female. Nests are typically domed back and built in a depression with the rim level with or slightly above ground. They are normally well concealed by a canopy of vegetation making them difficult to locate (Delaney 2000). Clutch sizes are commonly between three and six eggs per brood. This species is capable of multiple nesting attempts in one season. Eggs are smooth shelled, creamy white, and small in size (≈18 x 14 mm) with sparse markings concentrated at the large end of the egg. Egg incubation ranges from 11-13 days and is performed by the female. Nestlings generally fledge from the nest at 8-9 days post hatch. Offspring do not fly during initial departure from the nest, but move through vegetation on the ground (Rising 1996, Vickery 1996, Pyle et al. 1997, Beadle and Rising 2002). *Behavior*-Adult individuals are fairly secretive and draw little attention to territory boundaries and nest locations. When flushed from the nest, adults typically run a short distance before taking flight. Females also will use injury distraction displays to deter potential predators. Adults normally do not fly directly to the nest; they generally land several meters away and walk to the nest on the ground (Vickery 1996). Males normally perch and sing from perches at the periphery of the territory to avoid attracting attention to the nest location (Vickery 1996). Parental care, food provisioning and nest defense, is carried out by both genders. Nonparental nest attendants have been documented in this species and are thought to consist mainly of unrelated juveniles and neighboring adults (Kaspari and O'Leary 1988).

Morphology and Plumage-Grasshopper Sparrows are characteristically small to medium sized sparrows with relatively flat heads and large bills. Adult male and female individuals are unstreaked on the throat, breast, and flanks with brown and mottled coloration above. Back, rump, and tail feathers are streaked with chestnut, rust and black. Wings are mostly brown with feathers edged in pale brown; edge of wing is yellow at the carpel joint. The head has very distinctive brown lateral crown-stripes with a pale buffy-white median-crown stripe, yellow lores, pale yellow supercilium, and a grayish nape and side of neck. The tail is short with pointed retrices and bare shaft at the tip (Vickery 1996, Rising 1996, Beadle and Rising 2002). Juvenile individuals have a conspicuous band of streaks across the breast (Vickery 1996). The crown, back, rump, and nape are dark brown with feathers edged in pale buff or rusty brown (Beadle and Rising 2002).

Song-Male Grasshopper Sparrows typically sing two primary song types during the breeding season. These two primary song types are thought to function in mate attraction and territorial defense (Vickery 1996) in this species. The common alpha song consists of a high frequency insect-like vocalization with two or three introductory syllables followed by a high frequency trill. The Beta song is more musical than the alpha song consisting of a long sustained series of short buzzy syllables that vary slightly in pitch and frequency (Saunders 1935, Smith 1959). A third song type is normally confined to mated pairs and consists of a short descending trill sung by both genders to announce presence on the territory. Short staccato alarm calls are common in this species and are delivered by both genders (Saunders 1935, Smith 1959).

POPULATION/CONSERVATION GENETICS

The use of population structure data derived from genetic markers is based on the fact that polymorphic markers may be used to distinguish among individuals and populations (Smouse and Chevillon 1998). Developing conservation strategies that account for both demographic and genetic data at various scales may be critical in defining unambiguous evolutionary significant units (Avise 1994) necessary for effective management. Conservation and management plans based on ambiguous genetic population structure may accelerate declines or potentially lead to local extinction events (Hanski and Simberloff 1997).

The use of microsatellite DNA has become popular in studies examining fine-scale population structuring at various scales (Taylor et al. 1994, Paetkau and Strobeck 1994 Gibbs et al. 1997, Luikart et al. 1998). Typical microsatellite loci represent relatively short segments of highly variable DNA, sometimes referred to as a variable number of tandem repeats or VNTR's, that consist of repeat motifs of 2-6 nucleotides (i.e., GC, GTGT, GTTGTT, AGCAAGCA, etc.) that tend to occur in non-coding DNA (Weber and May 1989, Avise 1994). In some microsatellites, the repeated unit (e.g., GTT) may occur as many as 50-60 times. In diploid organisms such as birds and fish, each individual will possess two copies of any particular microsatellite segment (Avise 1994). With the advent of polymerase chain reaction (PCR) technology this property of microsatellite DNA was converted into a highly versatile genetic marker (Weber and May 1989).

To gain a more complete understanding of social behavior, reproductive success and kinship in birds, several studies have incorporated the use of reliable molecular genetic techniques (Gyllenstein et al. 1989, Westneat 1990). Single and multilocus DNA analyses of wild bird populations have the potential to reveal unexpected patterns of gene transmission (mating systems, parentage, and associations between individuals) resulting from diverse behavioral tactics employed by individuals (Delany et al. 2000). Accurate parentage and kinship assessment will contribute to a greater understanding of the genetic payoffs associated with behavioral strategies observed in the field.

Microsatellite markers present several advantages to parentage and kinship studies compared to allozymes, restriction fragment length polymorphisms (RFLP's), minisatellites, and randomly amplified polymorphic DNA (Queller et al. 1993). Recent studies have incorporated microsatellite loci to examine paternity (Houlden et al. 1996, Marshall et al. 1998, Adcock and Mulder 2002) and population assignment (Davies et al. 1999, Hansen et al. 2001) in animal studies.

Several newly developed estimators of pairwise relatedness coefficients using co-dominant genetic markers (Queller and Goodnight 1989, Goodnight and Queller 1999, Lynch and Ritland 1999, Wang 2002, Hardy 2003) have improved the efficiency and precision of studies focused on pedigree relationships. Hardy (2003) suggested that these estimators might be important tools for studies investigating sibship structure, isolation-by-distance in continuous

populations, kin selection, inbreeding and inbreeding depression, and for quantitative inheritance inferences in natural populations.

STUDY OBJECTIVES

The objectives of this study were to examine habitat requirements, mating success, parentage, nest site selection, and nest success of Grasshopper Sparrow (*Ammodramus savannarum pratensis*) populations colonizing reclaimed MTMVF complexes in southern West Virginia. Microsatellite DNA analysis of five polymorphic loci was used to resolve parentage, mating system type, and breeding success among individuals using the study sites. These data along with data derived using traditional ecological methodologies provide a quantitative description of preferred nesting habitat and overall reproductive success of these populations.

The specific objectives of this study include:

1. Determine if MTMVF grassland habitats are able to support breeding Grasshopper Sparrow populations (H_o: Grasshopper Sparrow nest survival on reclaimed MTMVF complexes does not differ from those on natural grassland areas);

2. Determine habitat requirements and/or preferences for nest site selection (H_o: All available habitat on mine sites is suitable for Grasshopper Sparrow nesting);

3. Develop and optimize molecular protocols and perform genetic analyses to assess overall population structure, mating system, parentage, and kinship of Grasshopper Sparrows (H_o: Grasshopper Sparrow mating systems are monogamous);

4. Determine if Grasshopper Sparrow assemblages on each mine complex are distinct breeding sub-populations or one large panmictic population (H_o: Grasshopper Sparrow populations on mine complexes are one large panmictic population).

STUDY AREAS

Study areas were located on the three MTMVF complexes in southwestern West Virginia (Fig. 1, Table 1.) that were investigated by Wood et al. (2001). The Cannelton mine was located on the border of Kanawha and Fayette counties in the Twentymile Creek watershed. This mine complex contained approximately 2180 ha of reclaimed grassland that ranged from 1 to 30 years in age. Dominant grassland vegetation included tall fescue (*Festuca arundinacea*), orchard grass (*Dactylis glomerata*), broomsedge (*Andropogon virginianus*), perennial ryegrass (*Lolium perenne*), birdsfoot-trefoil (*Lotus corniculatus*), mammoth red clover (*Trifolium pratense*) and sericea (*Lespedesa cuneata*). Dominant tree and shrub species on reclaimed areas included autumn olive (*Eleaganus umbellata*) and black locust (*Robinia pseudoacacia*). The study sites on this complex were Lynch Fork/Highwall (CLF) and Cabin Creek (CCC) (Fig. 2). The Lynch Fork site was the earliest mountaintop operation in West Virginia with post reclamation ages that ranged from 25 to 36 years. The CLF area included the Highwall site and also the Lynch Fork site (36.1 ha). These sites were combined to increase the area comprised of grassland greater than 20 years post reclamation. The CLF site contained large clumped patches of sericea, multi-flora rose, and autumn olive. Total ground cover of these three species on the Lynch Fork site was estimated to be 35-40%. Grasses and forbs dominated the Highwall site with less than 3% of ground cover being comprised of these three species. Reclaimed grasslands on the Cabin Creek site (28 ha) ranged in post reclamation age from 13-19 years. This site contained several small randomly distributed patches of sericea and multi-flora rose that accounted for approximately 10% of total ground cover. This site also contained several mature patches and hedgerows of autumn olive.

The Hobet 21 mine was located in the Mud River and Little Coal River watersheds in Boone County. This mine complex contained approximately 2431 ha of reclaimed grassland that ranged from 1 to 20 years in age. Dominant grassland vegetation on this complex included tall fescue, orchard grass, broom sedge, birdsfoot-trefoil, and mammoth red clover. Dominant tree and shrub species on reclaimed areas included autumn olive and black locust. The study sites on this complex were Adkins Fork (HAF) and Sugartree Branch (HST) (Fig.3). Post reclamation ages on the Adkins Fork site (51.3 ha) ranged from 11 to 15 years. This site contained several large clumped stands of autumn olive that comprised about 10% total ground cover and was also

heavily fragmented with gravel service roads. Sericea was present on this site, but at low numbers. The Sugartree Branch site (50.9 ha) contained grassland areas that ranged in age from 3-10 years post reclamation. This site had low densities of woody plants that accounted for less than 1% of total ground cover; however, small clumped stands of autumn olive were present.

The Daltex mine was located in the Spruce Fork watershed in Logan County. This mine complex contained approximately 1925 ha of reclaimed grassland that ranged from 1 to 20 years in age. Dominant grassland vegetation on this complex included tall fescue, orchard grass, broomsedge, birdsfoot-trefoil, mammoth red clover and sericea. Dominant tree and shrub species on reclaimed areas included autumn olive and black locust. The study sites on this complex were Rockhouse Creek (DRH) and Spruce Fork (DSF) (Fig. 4). The Rockhouse Creek site (45.1 ha) ranged in age from 13 to 20 years post reclamation and was heavily fragmented with service roads. This site contained moderate densities of sericea that comprised approximately 15% of ground cover and was planted with high densities of autumn olive and black locust (>20 stems/ha). The Spruce Fork site was the youngest site in the study with reclamation ages that ranged from 4-7 years. This area had very few woody stems and contained approximately 35% bare ground cover.

For comparison to the reclaimed mine study areas, a non-mined reference study area was established in the Mud River Wildlife Management Area (MRWMA), Lincoln County, near the Hobet 21 mine complex. This non-mined area consisted of an earthen dam that was constructed for recreational fishing purposes in this region approximately 10 years ago. The dam and associated flood plain areas comprised approximately 23 ha of early successional grassland habitat. The grassland site on the MRWMA also consisted of a mix of native and non-native forb and grass species including tall fescue, broom sedge, birdsfoot-trefoil, mammoth red clover and crown vetch (*Coronilla varia*). Additional grassland patches ranging in size from 2 to 12 ha were surveyed within the MRWMA to determine the presence of Grasshopper Sparrows. No individuals were detected on the smaller sized plots, so they were not continuously monitored in this study. I searched in the vicinity of the three mine complexes for additional non-mined grassland sites that supported grassland birds; however, none were found.

STUDY ORGANIZATION

The research findings are organized into three chapters. In chapter 2, I present data describing vegetative characteristics influencing habitat and nest site selection, nesting success, clutch size, and densities of Grasshopper Sparrows. In chapter 3, I use microsatellite markers to examine parentage, kinship, mating system, and overall genetic variation within and among Grasshopper Sparrow populations. Chapter 4 examines the efficacy of gender determination of Grasshopper Sparrows in the field. DNA gender determination methods are compared to standard morphological methodologies.

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Table 1. Location, size and age of six mountaintop mine / valley fill study areas surveyed in southwestern West Virginia, 2001-2002.

Mine	Watershed	Site Code	Stream Drainage	Study Site Area (ha)	Reclamation Age
Cannelton	Twentymile Creek	CLF	Lynch Fork/Highwall	36.1	20-36 years
		CCC	Cabin Creek	28.0	13-19 years
Dal-Tex	Spruce Fork	DRH	Rockhouse Creek	45.1	13-20 years
		DSF	Spruce Fork	40.0	4-7 years
Hobet 21	Mud River	HAF	Adkins Fork	51.3	11-15 years
		HST	Sugartree Branch	50.9	3-10 years

Figure 1. Study area locations for three mountaintop mining / valley fill complexes and one non-mined reference area in the Allegheny Plateau and Cumberland Plateau regions of southwestern West Virginia, 2001-2002.

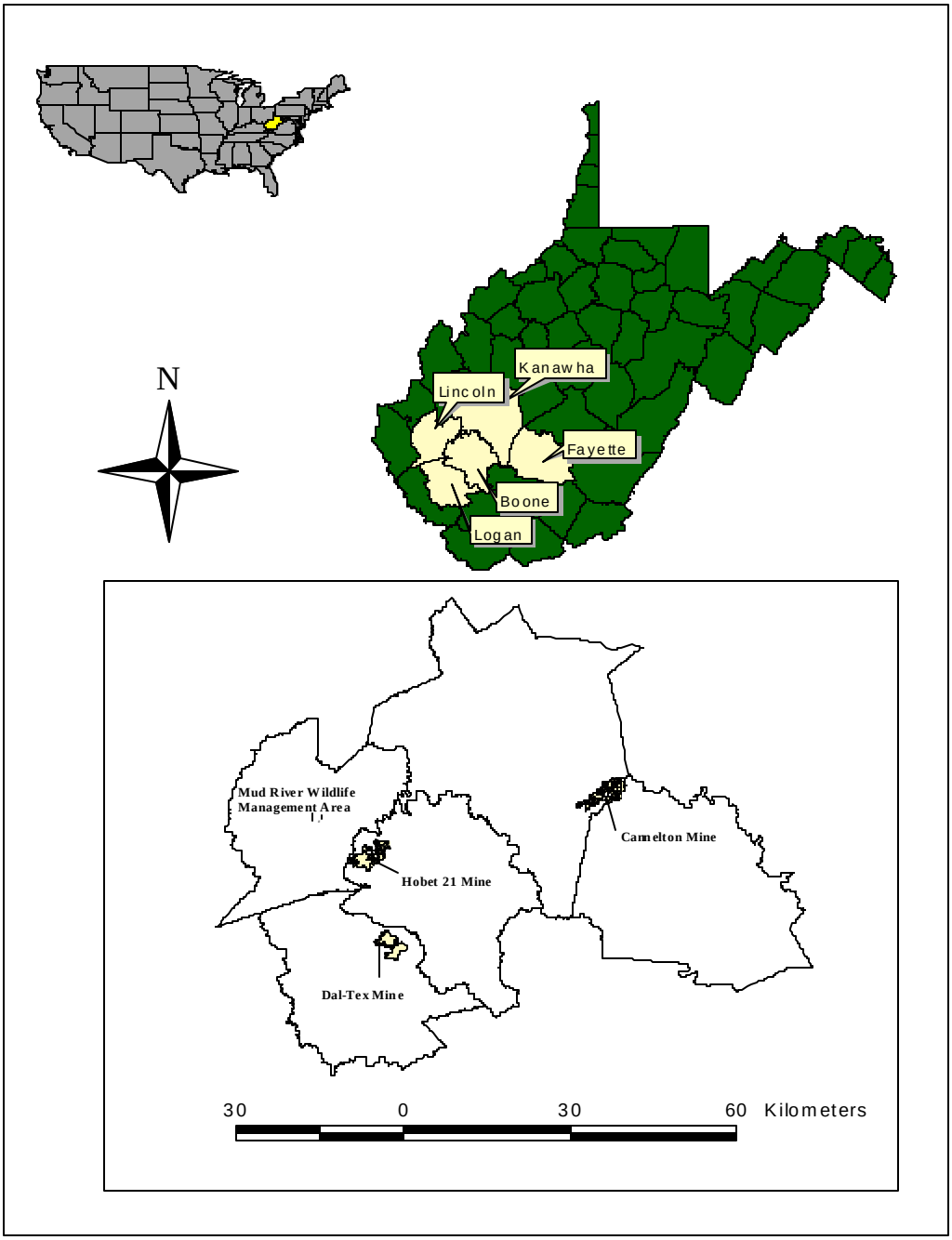


Figure 2. Location of study areas surveyed on the Cannelton mine complex. Reclamation age ranged from 11-20 years on the Cabin Creek site to 20+ years post reclamation on the highwall and Lynch Fork sites.

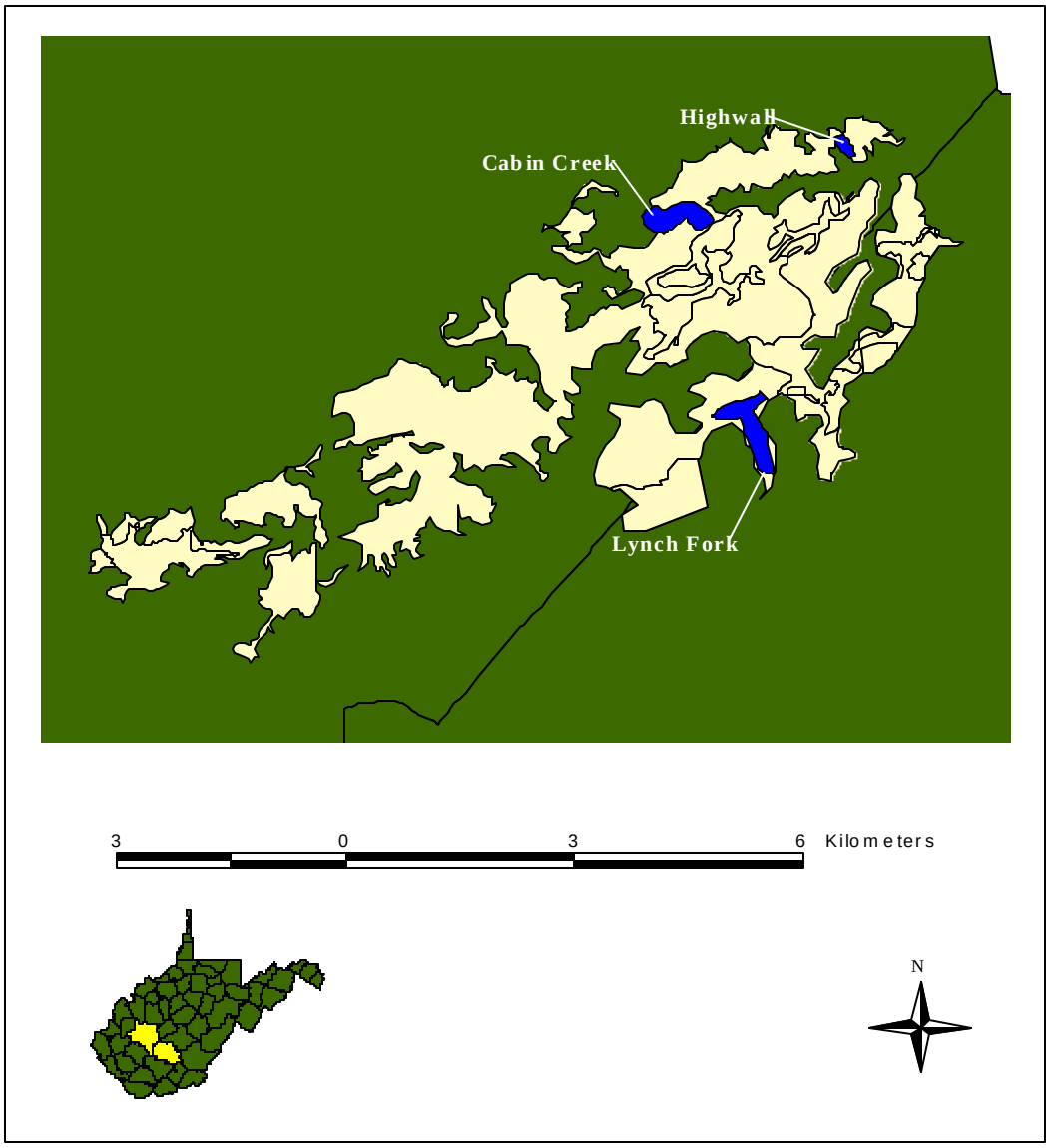


Figure 3. Location of the study sites surveyed on the Hobet 21 mine complex. Reclamation age ranged from 0-10 years on the Sugartree Branch site to 11-20 years Adkins Fork site.

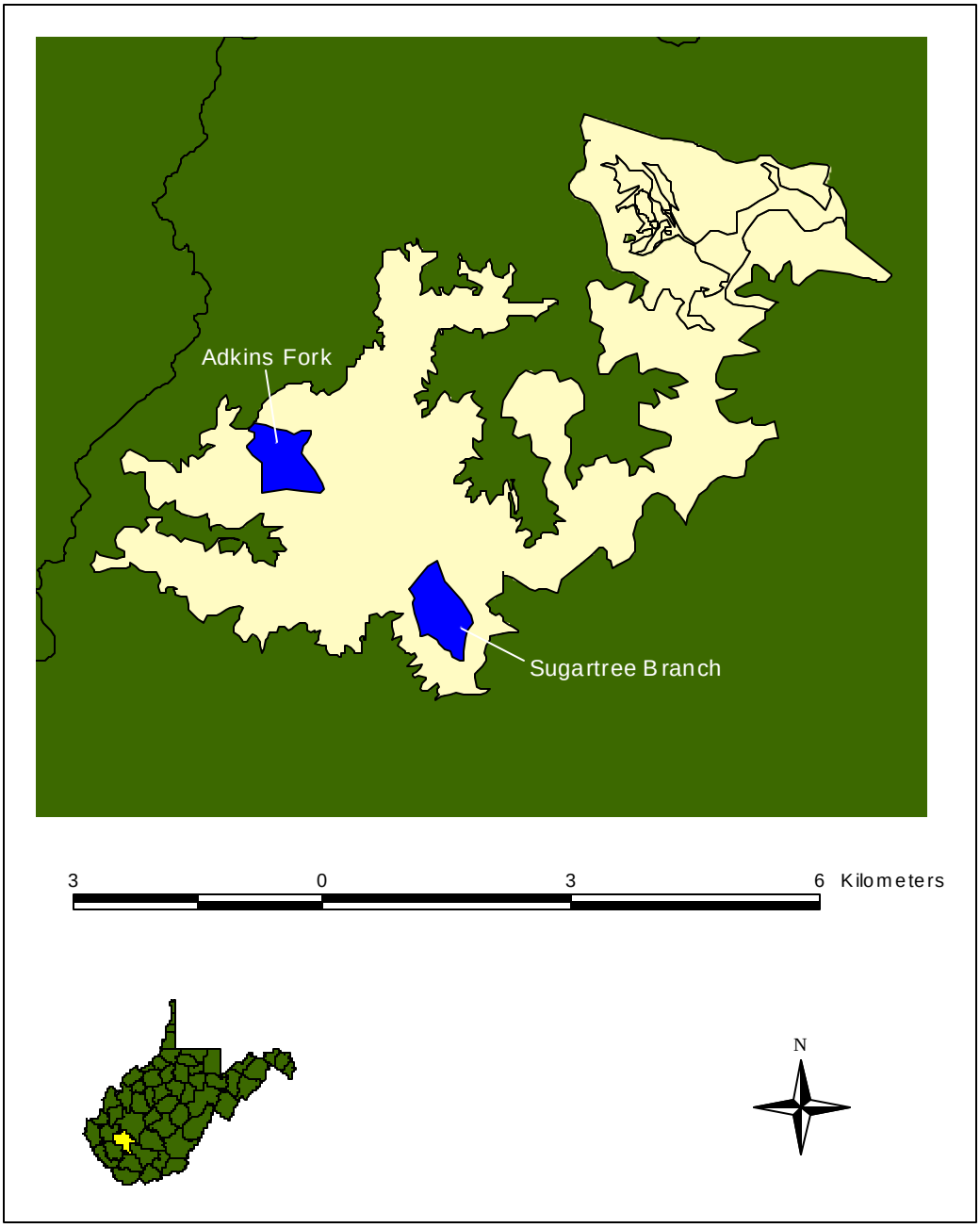
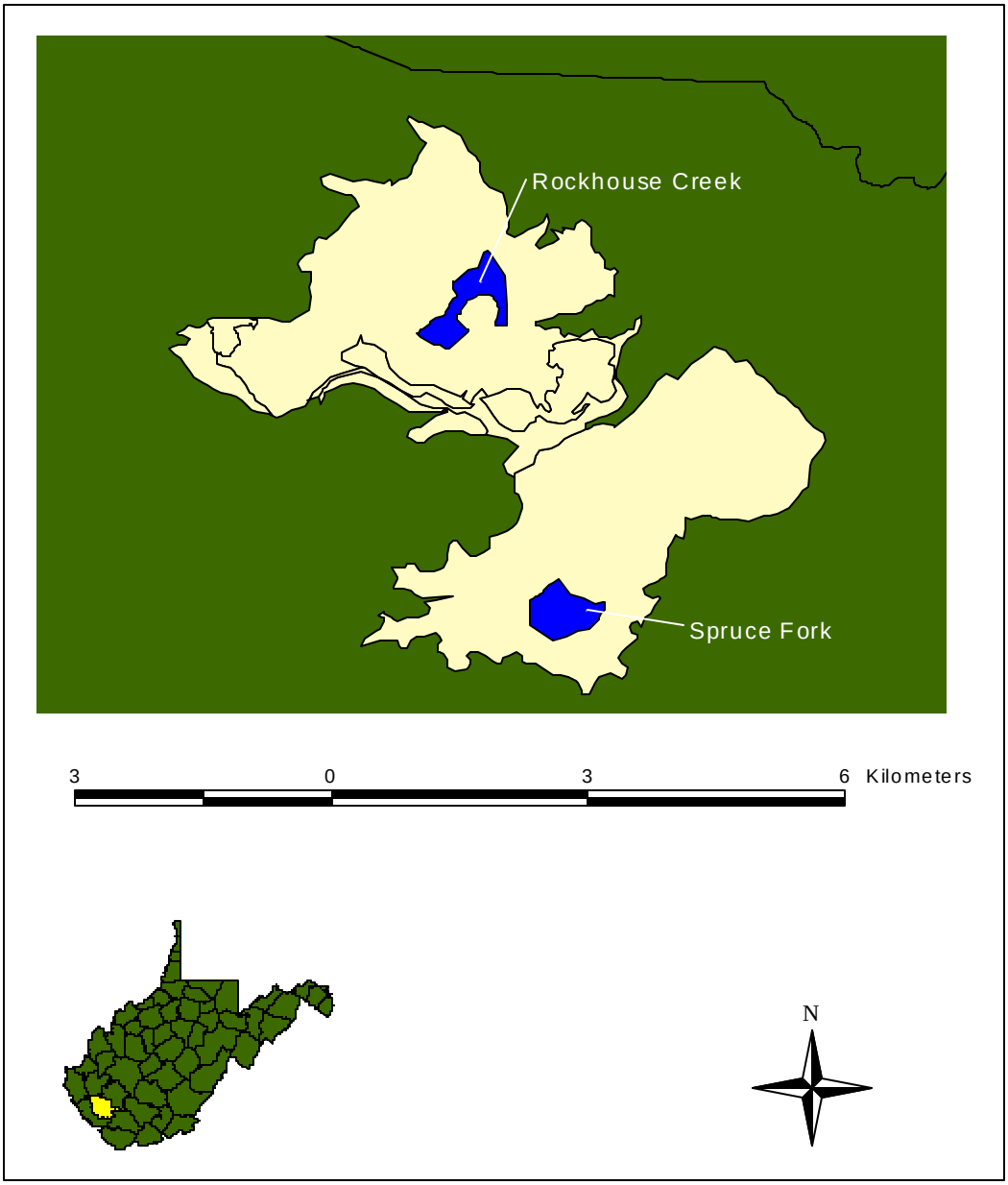


Figure 4. Location of the study sites surveyed on the Dal-Tex mine complex. Reclamation age ranged from 0-10 years on the Spruce Fork site to 11-20 years Rockhouse Creek site.



Chapter 2. Nest Survival and Habitat Selection of Grasshopper Sparrow Populations Breeding on Reclaimed Mountaintop Mines in West Virginia

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ABSTRACT

Mountaintop mining / valley fill (MTMVF) activities convert large areas of mature hardwood forest to early successional habitats creating potential habitat for grassland songbird populations. Three MTMVF mine complexes in southern West Virginia were examined to determine vegetation characteristics important to nest site selection and to estimate nest success for grasshopper sparrow populations inhabiting these sites. A total of 415 grasshopper sparrows were captured during the 2001-2002 breeding season. Systematic searches of study plots produced 75 active grasshopper sparrow nests. Nest survival for 2001-2002 breeding season (33%) is comparable to survival rates previously reported in the literature. Nesting success was higher at more recently reclaimed sites. Comparisons of habitat variables surrounding successful (n=37) and unsuccessful (n=38) nests indicate no significant differences in habitat variables except that nest substrate height (F=6.58, P=0.04) was greater at successful nests and dominant shrub width (F=6.63, P=0.06) was greater at unsuccessful nests. Comparisons of habitat variables measured at nests (N=37) and at fixed habitat plots (N=56) suggest differences in several of the ground cover estimates. Percent green and grass height at 1 m were lower at the nest plots while percent bareground, percent litter at 1 and 5 m from the nest, grass height at 3 m, shrub stem density, and Robel pole indices at the nest were higher at nest plots (P<0.10). Post reclamation age effects also were observed in comparisons of nest and habitat plots. With bare ground cover greatest on the 0-10 year plots, green ground cover greatest on the 11-20 year plots, and grass height at 1 and 3 m, litter depth at 1 and 5 m, and Robel pole estimates at 1 m greatest on 20+ year plots. Large reclaimed grassland habitats available on the MTMVF complexes are sufficient to support breeding populations of grasshopper sparrows; however, habitat will become unsuitable as succession occurs.

INTRODUCTION

Large-scale declines in migratory bird populations breeding in North America have become a major focus of studies addressing avian conservation, management, and biodiversity (Robbins et al. 1989, Askins et al. 1990, Terborgh 1992, Böhning-Gaese et al. 1993, Vickery and Herkert 2001). The exact causal mechanisms responsible for widespread population declines are still largely unknown due to trend variation across species ranges and because of limited data pertaining to dispersal dynamics within and among specific habitats (James et al. 1992, Donovan and Flather 2002). Fragmentation and degradation of breeding and wintering grounds, increased nest predation and brood parasitism coupled with increased edge are a few of the factors that have been associated with recently observed reductions in population number for many songbird species, including species dependent on grassland and early successional habitats (Robbins et al. 1989, Askins et al. 1990, Johnson and Temple 1990, Smith et al. 1992, Hoover and Brittingham 1993, Herkert 1994*b*, Warner 1994). The effects of multi-scale disturbance and habitat alteration on songbird abundance and nest survival have been well documented, especially in urbanized and agricultural landscapes (Vale and Vale 1976, Rodenhouse and Best 1983, Dickman 1987, Best et al. 1990, Bollinger et al. 1990, Bollinger and Gavin 1992, Suhonen et al. 1994, Warner 1994, Best et al. 1995, Germaine et al. 1998). However, studies investigating bird population declines of species dependent on grassland and early successional ecosystems have been limited until recently (Askins et al. 1990, Askins 1993, Herkert 1994*b*, Vickery and Herkert 2001).

A high number of grassland bird species have shown widespread and consistent population declines for more than thirty years (Robbins et al. 1989, Askins 1993, Herkert 1994). Results of several recent studies imply that intensification of agricultural practices, large-scale loss of suitable grassland habitat, inadequate management of remaining early successional habitats, disruption of natural disturbance regimes, invasion of woody and exotic vegetation, nest parasitism, and degradation of wintering grounds may be important factors influencing population dynamics in grassland systems (Kershner and Bollinger 1996, Herkert 1994, Johnson and Igl 2001, Vickery and Herkert 2001). A recent review of grassland bird research (Vickery and Herkert 2001) outlined several studies that examined a range of topics including habitat selection and population response to management practices (Bowen and Kruse 1993, Johnson 1996, Herkert and Glass 1999), nest success (Johnson and Temple 1990, Rohrbaugh et al. 1999),

nest parasitism (Hoover and Brittingham. 1993), and habitat area requirements (Vickery et al. 1994, Helzer and Jelinski 1999, Johnson and Igl 2001).

Studies investigating the use of surface mined land by bird populations have increased over the past few decades to examine how grassland and early successional species respond to these novel habitats. Most early studies were conducted on small surface mined lands that were either actively reclaimed or naturally revegetated through successional processes (Brewer 1958, Yahner and Howell 1975, Chapman 1977, Chapman et al. 1978, Crawford et al. 1978*a b*, Whitmore 1978, Whitmore and Hall 1978, Wray et al. 1978, Allaire 1979, Whitmore 1979, Wray 1979, Wackenhut 1980, Whitmore 1980, Strait 1981, LeClerc 1982, Wray 1982, Wray et al 1982). The majority of studies investigating avian use of small-scale surface mines suggest that birds follow a pattern of habitat use that is typical of what is seen in natural systems (Allaire 1980).

Mountaintop mining/valley fill (MTMVF) practices in West Virginia convert large areas of mature hardwood forest to early successional habitats creating potential habitat for grassland songbird populations. Mountaintop operations are large-scale area mines that target coal seams that cannot be economically mined with other conventional techniques. After coal extraction, much of the resulting overburden is replaced, regraded to approximate original contour, and replanted with native and exotic species compliant with the 1977 Surface Mining Control and Reclamation Act. Mountaintop mine reclamation processes in southern West Virginia create expansive grassland and early successional habitats in a region dominated by mixed mesophytic forest.

Data concerning species richness, nest site selection, and nest survival of birds using large MTMVF grasslands in the east are scarce (Wood et al. 2001). Several recently completed studies in southern Indiana (Bajema and Lima 2001, DeVault et al. 2002) examined breeding bird communities using large reclaimed surface mine sites (110-3200 ha); however, topography, climate, vegetation composition, and overall landscape structure in Indiana differs considerably from that in West Virginia. These differences in conjunction with the general lack of habitat suitability and productivity data for species colonizing MTMVF grasslands make assumptions about habitat selection and nest survival of songbirds breeding on large reclaimed grasslands in West Virginia difficult.

In a study of three MTMVF complexes in southwestern West Virginia during 1999 and 2000, Wood et al. (2000) found that Grasshopper Sparrows (*Ammodramus savannarum*) were the most common grassland bird species observed using grassland habitats. Nest densities, however, were generally low, particularly given the high abundance of singing males on the study areas. It may be that males were attracted to reclaimed areas but were not able to establish territories or to find mates. These questions need to be investigated further before decisions are made regarding the importance of these newly created grassland habitats to bird populations.

The objective of this study was to examine habitat requirements, nest site selection, and nest survival of Grasshopper Sparrow populations colonizing reclaimed MTMVF mine sites in southern West Virginia to determine if MTMVF grassland habitats are able to support breeding Grasshopper Sparrow populations. These data should provide a quantitative description of preferred nesting habitat and overall reproductive success of these populations.

METHODS

Study areas -Study areas were the three MTMVF complexes in southwestern West Virginia that were investigated by Wood et al. (2000) (Fig 1). The Cannelton mine (2180 ha) is located on the border of Kanawha and Fayette counties in the Twentymile Creek watershed, the Hobet 21 mine (2431 ha) is located in the Mud River and Little Coal River watersheds in Boone County, and the Dal-Tex mine (1925 ha) is located in the Spruce Fork watershed in Logan County.

For comparison to the reclaimed mine study areas, a non-mined reference study area (23 ha) was established in the Mud River Wildlife Management Area (MRWMA, 80 ha total grassland), Lincoln County, near the Hobet 21 mine complex. I searched in the vicinity of the three mine complexes for additional non-mined grassland sites that supported grassland birds; however, none were found.

Capture and Processing-Adult male and female Grasshopper Sparrows were captured on each study site with mist nets and conspecific song playback from April to August 2001 and 2002. All captured individuals were banded with U. S. Fish and Wildlife Service leg bands and a unique combination of two colored plastic leg bands for future visual identification. Basic physical information (gender, mass, age, wing chord measurements, bill width and length, tarsus

length and condition) was recorded. Gender and age of captured individuals was determined using plumage differences, physical measurements, and breeding characteristics (cloacal protuberance and brood patch) (Pyle et al. 1997). Offspring were captured prior to fledging (5-6 days post hatch) and banded with a USFWS leg band and a single colored leg band.

Nest survival-Nest searching was conducted on two 40-ha nest search plots in grassland areas of Hobet 21 (HAF & HST), Dal-Tex (DRH & DSF), and Cannelton (CLF & CCC) mine complexes and one plot on the MRWMA for a total of seven search areas (Appendix A-1). To obtain a good estimate of species-specific nest survival, a minimum of 20 nests must be monitored during a breeding season (Martin et al. 1997). Therefore, I set a yearly target of 25-30 nests for Grasshopper Sparrows nesting in the grassland habitat of the study sites. Field personnel trained in proper searching and monitoring techniques (Martin and Geupel 1993) searched each nesting area every 3-4 days. Nest searching began one-half hour after sunrise and concluded 8-10 hr later (approximately 0600-1600 EST). Nest searching methods followed national BBIRD (Breeding Biology Research and Monitoring Database) protocols (Martin et al. 1997). To control for search effort, nests were located by systematically searching study plots.

All nests found were monitored every 3-4 days (Martin et al. 1997) to verify activity. Because nests in grasslands are typically well concealed, they were marked for relocation using a staked flag placed a minimum of 15 m from the nest. We measured distance and bearing of the nest from the flag stake. Nests were monitored carefully to avoid disturbing or stressing the brooding female. When possible, nest searcher observations were conducted from a distance of no less than 15 m for up to 30 min to confirm that it was still active. Each nest was approached and checked for contents a minimum of four times throughout the breeding season: once when it was initially found, once to confirm clutch size, once to confirm brood size, and once to confirm fledging success or failure. Nests were not approached when avian predators (e.g., American Crows and/or Blue Jays) were observed nearby because these birds are known to follow humans to nests (Martin et al. 1997). Nest observers continued walking in a straight line away from the nest after checking contents to avoid leaving a dead-end scent trail that might be followed by mammalian predators (Martin et al. 1997). When directly observing nests, vegetation concealing the nest was moved to the side using a wooden stick to avoid transferring human scent to the nest and surrounding vegetation.

A nest was considered successful if it fledged at least one young. Fledging success was confirmed by searching the area around the nest for fledglings or for parent-fledgling interactions. However, if no fledglings were observed, the nest was considered to have fledged young if the median date between the last nest check when the nest was active and the final nest check when the nest was empty was within two days of the predicted fledging date (Martin et al. 1997). In this case, the number of fledglings assigned to the nest was the number present during the last nest check.

Nest survival was calculated using the Mayfield method with daily nest survival estimates calculated separately for the incubation and brooding periods because there may be differential nest survival between these two periods (Mayfield 1961, Mayfield 1975). Overall daily survival rates were calculated as the product of incubation and brood daily survival. Survival during the egg-laying stage was not included in the calculation of overall nest survival because few nests were found during this stage of the nesting cycle. To increase sample size, account for temporal variation, and to better examine effects of reclamation age on nest survival, data from nests monitored during the 1999-2000 (N=19, Wood et al. 2000) breeding seasons were combined with those of the 2001-2002 breeding seasons (N_{Total}=94). Nest survival rates among mine complexes, among post reclamation ages, and between nests with and without helpers were compared with the software program CONTRAST (Hines and Sauer 1989). This software was designed for multiple comparisons of survival rates using chi-square methods that incorporate associated variance and covariance estimates. Critical values were adjusted to account for multiple tests of the same hypothesis using the Bonferroni adjustments (Rice 1989).

Vegetation sampling-After a nest fledged or failed, vegetation within an 11.3 m radius circle surrounding the nest was sampled to determine vegetation characteristics important to nest site selection and overall nest survival. An additional eight fixed vegetation sampling subplots were systematically selected and surveyed on each search plot (N=56) to examine differential nest site selection in this species. We measured vegetation for each nest monitored using methods modified from James and Shugart (1970) and the Breeding Bird Research Database program (BBIRD; Martin et al. 1997).

Percent ground cover estimates in nine categories (green, grass/sedge, shrub/seedling, moss, bare ground, forb/herbaceous, woody debris, litter, and water) were estimated using an

ocular sighting tube (James and Shugart 1970). Twenty ocular-tube readings were recorded on each sample plot every 2.26 m along four 11.3-m transects that intersected at the center of the sample plot (Fig. 2). Total ground cover for each sample plot was the sum of the sight tube measurements in each category divided by 20. Vegetation species occurrence also was measured with twenty ocular-tube readings. The species visible in the crosshair of the sight-tube was identified and recorded. Species occurrence estimates for each sample plot was the sum of the sight tube measurements divided by 20. Habitat variables and sampling methods are described further in Appendix A. 1.

A Robel pole (Robel et al. 1970) was used to calculate an index of vegetative cover and an index of biomass (Kirsch et al. 1978) (Fig. 3). Measurements with a Robel pole have been widely used to characterize vegetation near bird nests (Kirsch et al 1978), and to measure the height of vegetation to provide an index of biomass (Robel et al. 1970). To quantify vegetative cover, measurements with the Robel pole were taken at the sample plot or nest center, and at 1, 3, and 5 m along each transect for a total of 13 measurements (Fig. 2). Vegetative cover at an individual plot is the average of these 13 measurements. The four visual obstruction measurements at each distance were averaged to examine microhabitat characteristics related to cover. Grass height and organic litter layer depth were measured at 17 locations along the four transects: at the center and at distances of 1 m, 3 m, 5 m, and 10 m along each transect (Fig. 2).

Additional nest characteristics were measured to examine differences among individual nests. These included percent slope, slope orientation, nest height (cm), width and depth of nest rim and cup (cm), nest substrate height (vegetative and reproductive), and distance to foliage edge.

All tree and shrub stems present within the 11.3 m nest and habitat plots were counted, identified to species, and placed into 1 of 2 size classes: 2.5 cm and > 2.5 but <10 cm in diameter. Percent woody cover present was visually estimated for the entire 11.3 m sample plot. For the largest tree and shrub on the plot, I measured height, width of the overall plants, and distances to center within the 11.3 m nest and habitat plots.

Statistical analyses-Habitat variables were tested for differences among sample plot types (nests vs. habitat plots) and also between successful and unsuccessful nests using analysis of variance (ANOVA, Zar 1999). Main factors in the use vs. available habitat model included sample plot

type, mine, year, post reclamation age, and mine with plot type by year and plot type by reclamation age included as interaction terms. Main factors in the nest success model included nest success/failure, plot, year, post reclamation age, and mine with sample plot type (success/failure) by year and sample plot type by reclamation age included as interaction terms. I also compared habitat variables on mined and non-mined plots measured in 2002. Main factors in the land use model were sample plot type (mined vs. non-mined), and site (each of the three mines and the MRWMA). Vegetative species occurring on sample plots were examined for differences to identify species important in habitat selection of Grasshopper Sparrows. These species comparisons were made with the ANOVA models described above for use vs. available, nest success, and land use models. The mean values for all variables from the vegetation sample plots were used in the analyses.

Grasshopper Sparrow abundances were examined for differences between years and mine complexes with analysis of variance (Zar 1999). The main factors in the model were year, mine, and plot nested in mine, with year by mine included as an interaction term. The Waller-Duncan K-ratio t-test was used to examine abundance relationships among mine complexes. Abundances also were examined for differences among reclamation ages. The main factors in the model were year, reclamation age, and mine complex, with year by mine included as an interaction term.

Chi-square procedures were used to examine preferences in nest substrate selection characterized by reclamation age (Zar 1999). Substrate species were grouped to increase sample size within categories to meet minimum sample size requirements of chi-square.

Clutch size and the number of fledged offspring were examined for differences between mine sites, month of nest initiation, and year with Poisson regression analysis to generate Wald Chi-square statistics (GENMOD; SAS Institute Inc. 1999). The main factors in both models were initiation month, mine site, reclamation age, and year, with mine site by month included as an interaction term.

Slope aspects were transformed before analyses with the Beers et al. (1966) procedure, using the equation: A' = (COS (45-A)+1), where A' is the transformation index and A is the direction the slope faces in degrees (Frazer 1992). With this transformation, northeastern facing slopes receive a value of 2 and reflect mesic conditions, while southwestern exposures receive a

value of 0 and reflect xeric conditions. Other exposures are distributed between these values (Frazer 1992).

Variables that were not normally distributed were appropriately transformed prior to analysis (Zar 1999). All percentage variables (i.e. slope, ground cover, and green) were transformed using the arcsine-square root transformation (Zar 1999). Stem densities were transformed using the transformation $X'=\log_{10}(X+1)$, where X' is the transformed value and X is the original value (Zar 1999). Although most habitat variables were not normally distributed after transformation, I proceeded with ANOVA because it is robust to deviations from normality (Zar 1999).

All analyses were conducted using the SAS software package (SAS Institute Inc. 1999). Differences were considered significant at $\alpha = 0.10$ to account for variation or trends that may have biological significance. Askins et al. (1990) suggested that it is better to use liberal rejection levels when dealing with conservation and management issues.

RESULTS

Grasshopper Sparrow density-A total of 415 Grasshopper Sparrows were captured, banded, and processed on the study sites during the 2001 and 2002 breeding seasons. Mist netting effort averaged over all reclaimed sites resulted in a capture rate of 0.20 individuals per net hour (Table 1). Juveniles that were captured and banded in and around nests were not included in the mist net capture effort calculations.

Grasshopper Sparrow abundance on the mine complexes ranged from 0.69 individuals/ha on the Cannelton mine to 0.92 individuals/ha on the Hobet 21 complex (Table 1). Significant differences were detected in total abundance among the mine complexes (F=12.56, d.f.=2, P=0.03). Further analyses with the Waller-Duncan K-ratio t-test show a significant difference in abundance between the Cannelton and the Hobet 21 mines; however, no differences were detected between the Dal-Tex mine and the other two mine complexes. No differences were detected in abundance by year (F=5.16, d.f.=1, P=0.96) and no interaction was detected between year and mine (F=3.96, d.f.=2, P=0.14). Grasshopper sparrow abundance on the MRWMA site was 0.30 individuals/ha. Additional sites on the MRWMA were surveyed, however, no Grasshopper Sparrows were located on these sites. Grasshopper Sparrow abundances were

examined according to post reclamation age with abundances ranging from 0.60 individuals/ha on the 20 + years post reclamation site to 0.97 individuals/ha on the 0-10 year post reclamation sites (Table 2). No differences were detected in reclamation age comparisons (F=0.65, d.f.=2, P=0.46), and no interactions were detected between year and mine (F=3.44, d.f.=5, P=0.13).

An additional 76 non-target individuals were captured on the study plots with the most common species including American Goldfinch, Eastern Meadowlark, Field Sparrow, and Indigo Bunting (Table 3). Non-target individuals were examined immediately after capture to determine gender and age and then released.

Nest density-Systematic searches of study plots in 2001 and 2002 produced 75 active Grasshopper Sparrow nests on the three mines surveyed. Overall nest search effort resulted in one nest found per 7.93 hours of effort for all sites combined. Nests located off of the study plots (N=4) were not included in search effort calculations because they were not located by systematically searching study areas. An additional 18 nests (4 Horned Lark, 1 Field Sparrow, 2 Killdeer, 1 Savannah Sparrow, and 10 Eastern Meadowlark) of non-target species were located on the surveyed mine sites but were not continually monitored due to time and personnel constraints.

Nest densities on the mines ranged from 0.14 nests/ha on the Hobet 21 mine to 0.16 nests/ha on the Cannelton and Dal-Tex mines (Table 4). No nests were found on the MRWMA site. Nest density by post reclamation age ranged from 0.07 nests/ha on the 20 + years post reclamation site to 0.16 nests/ha on the 11-20 year post reclamation sites (Table 5).
Nest structure and substrate.-The most common primary nest substrates identified for all Grasshopper Sparrow nests during the 2001-2002 breeding seasons were grass species (Table 6). I found that 89% of all monitored nests occurred in tall fescue (*Festuca arundinacea*), broom sedge (*Andropogon virginianus*), orchard grass (*Dactylis glomerata*), and perennial ryegrass (*Lolium perenne*). Secondary and tertiary nesting substrates comprising the nest clump were identified to species (Table 7). Ninety-one percent of all monitored nests were associated with some secondary substrate, while only 37% of nests were built in clumps comprised of more than three vegetative species.

All monitored nests were of cup design built into a slight depression in the ground and domed at the back. Overhanging vegetation with mean coverage of approximately 84%

concealed all nests. Nests were constructed from small stems and grasses and lined with fine grass and hair. Mean nest rim height was 1.54 ± 0.13 cm from ground level with an average nest rim width of 1.81 ± 0.05 cm. Mean nest depth was 6.10 ± 0.12 cm, with a mean nest inside width of 6.52 ± 0.08 cm. Mean nest clump area was 1564 ± 132 cm^2 for all nests monitored.

Clutch Size and Productivity-Grasshopper Sparrow mean clutch sizes during the 2001 and 2002 (N=75) breeding seasons ranged from 3.25 to 5.00 eggs/nest with an overall mean of 3.77 ± 0.09 (Table 8). Productivity (individuals fledged/nest) ranged from 0 to 5.00 with an overall mean of 1.81 ± 0.22 (Table 8).

To increase sample size for examining effects of reclamation age, clutch size and productivity data from nests found during the 1999 and 2000 (N=19, Wood et al. 2000) breeding seasons were combined with those from the 2001 and 2002 breeding seasons. Mean clutch size ranged from 3.60 ± 0.70 on the 20+ year site to 4.03 ± 0.10 on the 11-20 year sites and did not differ among reclamation ages (Wald χ^2=1.03, d.f.=2, P=0.36; Table 9). Significant differences in clutch sizes were detected for month of initiation (Wald χ^2=36.13, d.f.=3, P<0.001) with larger clutch sizes observed early in the breeding season (Table 10).

Mean productivity ranged from 1.67 ± 0.24 individuals fledged/ nest on the 11-20 year post reclamation sites to 2.3 ± 0.36 individuals fledged/ nest on the 0-10 year post reclamation sites (Table 10). No differences were detected in comparisons of number fledged by year (Wald χ^2=3.66, d.f.=3, P=0.30) or month of initiation (Wald χ^2=5.67, d.f.=3, P=0.13) (Table 10).

Nest survival.-An overall nest survival estimate for 75 Grasshopper Sparrow nests monitored during the 2001 and 2002 breeding seasons was 33%. To increase sample size and to better examine effects of reclamation age on nest survival, data from nests collected during the 1999-2000 (N=19, Wood et al. 2001) breeding seasons were combined with those of the 2001-2002 breeding season (N_{total}=94). Nest survival rates that were classified by mine complex (N=94) ranged from 28% on the Hobet 21 mine (N=34) to 48% on the Dal-Tex mine (N=36), with an overall estimate of 35% on all mines combined (Table 11). Survival rates differed among mine complexes (χ^2=203.3, d.f.=2, P<0.001). The Dal-Tex mine had higher nest survival rates than both the Hobet 21 mine (χ^2=167.5, d.f.=1, P<0.001) and the Cannelton mine (χ^2=105, d.f.=1,

$P<0.001$). No differences were detected in survival rates between the Hobet 21 mine and the Cannelton mine ($\chi^2=1.74$, d.f.=1, $P=0.19$).

Overall nest survival rates grouped by reclamation age class ranged from 13% on the 20+ year post reclamation site to 43% on the 0-10 year post reclamation sites (Table 12). Nest survival rates differed among reclamation age classes ($\chi^2=145.2$, d.f.=2, $P<0.001$). Nest survival in the 0-10 year age class was significantly greater than in the 11-20 post reclamation class ($\chi^2=81.46$, d.f.=1, $P<0.001$) and also greater than the 20+ year age class ($\chi^2=90.72$, d.f.=1, $P<0.001$). Differences in survival rates also were detected between 11-20 year and 20+ year post reclamation classes ($\chi^2=41.21$, d.f.=1, $P<0.001$).

Nest failures were attributed to several different factors including weather, fire, human disturbance, and predation. Nest predation accounted for 76% of 45 nest failures on the mine complexes. Potential predator species that were sighted on the mine complexes are listed in Table 13. Sixteen percent of nest failures were directly caused by human disturbances on the mines. Removing these nests from survival calculations increased nest survival estimates by 3-4% in each reclamation age class (Table 12). Five nests were crushed by recreational all terrain vehicles, while two nests failed due to fires intentionally set by vandals. Seven percent of nest failure was attributed to high rainfall and flooding conditions during the 2001 breeding season. One failed nest containing an adult female and three nestlings was found trampled, presumably by a white-tailed deer.

Nest helpers-Nest helpers were observed at five nests during the 2001 breeding season and at six nests during the 2002 breeding season. All instances of nest helping occurred on less than 20 years post reclamation sites. The majority of observations consisted of food delivery and subsequent transfer of the food item to a brooding female by an individual other than the paternal parent. All food transfers occurred on the ground within 10 m of the nest. Unique color band combinations were used to identify individuals on specific territories. On all territories, one banded male perched, vocalized, and actively defended the territory while food transfers took place. Aggressive behavior was observed between the defending male and conspecific intruders; however, no aggression was observed between the defending male and the nest helpers. Mayfield nest survival rates were 50% (variance=2%) for nests with confirmed helpers (N=11) and 32% (variance=0.6%) for nests without helpers (N=83).

Habitat characteristics-Only two habitat variables differed between successful (N=37) and unsuccessful (N=38) nests (Table 14). Reproductive nest substrate height at the nest was greater at successful nests and dominant shrub width greater at unsuccessful nests. No differences were detected in vegetative species abundance surrounding successful and unsuccessful nests (Table 15).

Several habitat variables differed between nests (N=75) and fixed habitat plots (N=96) (Table 16). Nest plots had greater litter cover, litter depth at 1m and 5m, bare ground, Robel pole estimates at the nest, grass height at 3 m, and shrub stem density, than habitat plots. Habitat plots had greater percent green estimates and grass height at 1m than nest plots. Vegetative species occurring at nests and the fixed habitat plots were different for several of the species surveyed (Table 17). Broomsedge and sericea abundances were greater on habitat plots, while golden rod and sweet clover were most abundant at nest plots.

At nest plots, which reflects habitat conditions where Grasshopper Sparrows chose to place their nests, reclamation age differences were only observed in grass height at 3 m with greatest values occurring on the 20+ year aged plots (Table 18). Reclamation age effects were observed in abundance comparisons of tall fescue, alfalfa and sweet clover (Table 19).

Some habitat characteristics differed among post reclamation age classes using data from nests and habitat plots. Bare ground cover was greatest on the 0-10 year plots, percent green ground cover was greatest on the 11-20 year plots, and grass height at 1 and 3 m, litter depth at 1 and 5 m, and Robel pole estimates at 1 m were highest on 20+ aged plots (Table 20). Percent woody cover was highest on 11-20 year plots while dominant shrub height and width was less on the 20+ year aged plots. Age differences were also observed in species abundance comparisons. Mammoth red clover and alfalfa abundances were highest on 0-10 year plots; broomsedge and sweet clover were highest on the 11-20 year plots, while tall fescue, sericea, and aster spp. were highest on the 20+ year aged plots (Table 21).

Habitat variables also were compared among mined and non-mined grasslands that were sampled during the 2002 breeding season. Grass ground cover and grass height at 1 and 10 m were significantly greater on the non-mined areas (Table 22). Forb ground cover was less on non-mined areas. Vegetative species abundance on non-mined and mined grasslands differed for two species (Table 23). Tall fescue abundance was significantly higher on the non-mined areas, while birdsfoot-trefoil abundance was higher on the mined grasslands.

DISCUSSION

Several grassland species including Grasshopper Sparrows, Eastern Meadowlarks, Horned Larks, and Savannah Sparrows were confirmed breeding on the MTMVF complexes in southern West Virginia. While all of these species were detected at relatively high frequencies, Grasshopper Sparrows were the most abundant species on these sites (0.69-0.92 birds/ha). Grasshopper Sparrows are confirmed breeders in a wide range of habitat types including native prairies, old fields, pastures, airports, agricultural fields, and reclaimed surface mines (Whitmore 1978, Wray et al. 1982, Vickery 1996, Kershner and Bollinger 1996, Koford and Best 1996, Koford 1999, Rohrbaugh et al. 1999, Warren 2001, Ingold 2002). This species appears to be opportunistic and will colonize suitable grassland habitats when available. Reclaimed grasslands on MTMVF complexes are expansive "islands" of grassland habitat that were historically unavailable to grassland dependent species.

Low recapture rates (≈3%) on the study areas suggest that site fidelity to specific natal areas is low in migratory Grasshopper Sparrow populations. While few individuals returned to specific natal areas, 28 individuals that were color banded during the 2001 season (≈14%) were observed on non-sampled areas of the mine complexes in 2002. All recaptures and color-banded individuals were observed on the mine complexes in which they were originally captured, implying some degree of fidelity to the mine area.

Grasshopper Sparrows selected grassland habitats containing a mix of cultivated and invading native and non-native grass and forb species interspersed with patches of bare ground. Wiens (1969) and Whitmore (1981) investigated vegetation structural characteristics comparing vegetation within Grasshopper Sparrow nesting territories to vegetation outside of territories. Wiens (1969) found that vegetation density, litter depth, and forb height and density were greater outside of territories. Similarly, Whitmore (1981) reported that territories were more sparsely vegetated with low vegetation heights, low forb, grass, and shrub covers, but a high percentage of bare ground. In the comparison of used vs. available habitat on the mine complexes, I found that Grasshopper Sparrows preferred nesting habitats comprised of a moderately deep litter layer, a high degree of vertical structure at the nest, and a high percentage of bare ground cover near the nest. Grass heights in nesting territories on MTMVF complexes are similar to those reported

in pastures and hayfields in Wisconsin (Sample 1989) and on those described on small reclaimed mines in northern West Virginia (Whitmore 1981).

Grasshopper Sparrow nests were not found on grassland areas that were newly reclaimed or sparsely vegetated. Non-colonized areas were characteristically comprised of sparse grass and forb communities, little vertical structure, no litter layer, and a high degree of bare ground and exposed rock. Breeding pairs were observed colonizing and nesting in established reclaimed grassland areas that were greater than five years post reclamation.

Monitored nests were cup shaped, domed along the back, and constructed in the base of a dense vegetation clump with a small area of bare ground near the nest opening. All nests were constructed of a variety of woven grasses, forbs, and small stems with a distinct inner lining made of fine grasses and hair. Observations of nest construction in this study are similar to those previously reported (Pyle et al. 1997, Rising 1996, Delany 2000, Beadle and Rising 2002). Nests were built primarily in clumped grass species; however, forb species were selected as the primary substrate for 11% of the surveyed nests. Nests were well concealed by surrounding and overhanging vegetation that may function to regulate nest microclimate and reduce predation risks.

I found that ground cover and grass height increased with age, while the amount of bare ground available for foraging and mating decreased. Successional changes to vegetative structure on older unmanaged mined grasslands may influence habitat suitability for this species. Wray et al. (1982) found that abundance decreased over time as vegetation changes resulted in habitat that was less than optimal.

Greatest nest success occurred in the youngest reclamation age class (0-10) and decreased with reclamation age. Although nest survival on the 11-20 year old sites (34%) was significantly lower than that of the 0-10 year old sites, it was within the range found in other studies of Grasshopper Sparrows. Nest survival estimates from this study are comparable to those reported on Conservation Reserve Program fields in North Dakota (Koford 1999), and tall grass prairie in Oklahoma (Rohrbaugh et al. 1999), lower than those reported on Conservation Reserve Program fields in Missouri (McCoy et al. 1999) and on surface mines in Ohio (Ingold 2002), and higher than those reported on waterfowl production areas in North Dakota and surface mines in northern West Virginia (Wray et al. 1982).

Nest densities on the 11-20 year post reclamation age grasslands (0.16/ha) were similar to densities on the 0-10 year post reclamation age grasslands (0.15/ha) suggesting a preference for the vegetative structure, composition, and characteristics associated with these younger age classes. Grasslands in these age classes typically consisted of mixed grass and forb communities dominated by broomsedge, tall fescue, orchard grass, birdsfoot-trefoil, mammoth red clover, and alfalfa. These grasslands contained few woody stems, but contained a high degree of bare-ground patches and exposed rock. Lowest nest densities on the 20+ post reclamation aged areas lend support to studies that proposed avoidance of grassland areas with dense cover and established or invading tree or shrub communities (Smith 1963, Wiens 1969, Whitmore 1979). Nest densities on the mine complexes are similar to those reported in tall grass prairies in Oklahoma (Rohrbaugh et al. 1999), lower than those reported on surface mines in Ohio (Ingold 2002), and much higher than those reported on surface mines in northern West Virginia (Wray et al. 1982) and those previously reported on these mines (Wood et al. 2001) (Table 24).

Clutch sizes of Grasshopper Sparrow nests monitored on the MTMVF complexes (overall 3.77 ± 0.09) conform to those previously reported in the literature for this species (Wray et al. 1982, Ehrlich et al. 1988, Rising 1996, Pyle et al. 1997, Delany 2000b, Beadle and Rising 2002). Clutch sizes decreased significantly as the breeding season progressed, however, no differences were observed in the number of offspring fledged. Decreases in clutch size over the breeding season may be attributed to several factors including, decreased day length, reduced reproductive endocrine cycles, reduced food supplies, and high mid-summer temperatures resulting in reduced feeding rates (Wray et al. 1982). Wray et al. (1982) further suggested that decreases in clutch size over the breeding season may be related to decreasing metabolic resources in females most likely due to double and triple brooding practices in this species.

Predation events accounted for the majority (76%) of nest failure on the mine complexes. Wray et al. (1982) proposed that reclaimed surface mines and adjacent areas might harbor a diverse assemblage of potential nest predators. I directly observed 17 predatory species on or near the study sites, with the most commonly encountered species being the Black Rat Snake (*Elaphe obsoleta obsoleta*), American Crow (*Corvus brachyrhynchos*), and *Peromyscus* spp. Numerous potential predator species that may contribute to predation events impacting Grasshopper Sparrow survival have been documented on my study areas by Balcerzak (2001), Chamblin (2002), and Williams (2003).

Several studies suggest that snakes are responsible for a large number of predation events of grassland bird nests (Best 1978, Gottfried 1978, Morrison and Bolger 2002). However, additional research is needed to determine which predators are responsible for Grasshopper Sparrow predation events documented on MTMVF complexes. White-tailed deer also have been implicated as a potential grassland nest predator (Pietz and Granfors 2000). Pietz and Granfors (2000) used camera systems to directly observe depredation events on several ground nesting species, including Grasshopper Sparrows. While I did not directly observe depredation events by white-tailed deer, we did have a nest fail due to trampling presumably by a deer. This nest contained an adult female and three nestlings that were found trampled in the nest.

Human interference and disturbance were directly responsible for 16% of nest losses on the mines. Older reclaimed areas are commonly used for recreational purposes because the resulting post-mined landscape is attractive for off-road vehicle use. Overall nest survival rates increased 3% to 4% when nests lost to human failure were removed from the analysis. Weather was a relatively unimportant factor except for three nests that failed due to a prolonged period of heavy rain during the 2001 breeding season.

Brood parasitism by Brown-headed Cowbirds did not impact Grasshopper Sparrows nesting on our study areas even though several cowbird females were observed foraging on the sites. I found no evidence of parasitism in the 94 Grasshopper Sparrow nests found and monitored on the mine complexes. The probability that brood parasitism occurred without detection is low, because smaller passerines normally do not eject foreign eggs and because abandoned nests due to parasitism would have been detected by observers (Morrison and Bolger 2002).

Nest helpers were observed at 11 different Grasshopper Sparrow nests monitored during the 2001-2002 breeding seasons. Observed behaviors consisted mainly of food delivery and transfer from a non-parental individual to the brooding female. No food transfers were observed between nest helpers and the paternal parent. Altruistic behavior by non-parental nest attendants has been previously described in this species (Kaspari and O'Leary 1988). To gain a better understanding of altruistic behavior in this species, genetic studies may be designed to investigate the relatedness of the individuals participating in these behaviors. Brown (1987) proposed that species displaying these behavioral adaptations are often sedentary with some type of constraint that negatively impacts independent reproduction. However, recent studies

investigating this phenomenon have documented helping behavior in several migratory passerine species including Bobolinks (Beason and Trout 1984), Hooded Warblers (Tarof and Stutchbury 1996), and Ovenbirds (King et al. 2000). Cooperative breeding behavior has been described in several North American sparrow species including Brewer's Sparrow (Gill and Krannitz 1997), Chipping Sparrow (Middleton and Prescott 1989), and Henslow's Sparrow (Guzy et al. 2002), all of which are migratory.

The lack of large natural grassland areas in this region made it difficult to examine the relationships among mined and non-mined habitat. Grasshopper Sparrows were present on the MRWMA site later in the summer; however, densities were much lower than on the mine complexes. Several additional smaller grassland areas (1-10 ha) were surveyed on the MRWMA, but no Grasshopper Sparrows were detected using these areas. Low densities on this site may be related to patch size and area sensitivity in this species (Herkert 1994 *a b*, Vickery et al 1994, Helzer and Jelinski 1999, Walk and Warner 1999, Winter and Faaborg 1999, Johnson and Ingl 2001) rather than to differences in vegetative composition and structure.

Conservation implications-Management recommendations for MTMVF complexes should include a suite of management prescriptions that are implemented in a rotational manner. Managed grassland habitat areas should be large enough to attract and support breeding grassland bird populations. Several studies suggest that Grasshopper Sparrows may display some degree of area sensitivity and require larger grassland patches (Herkert 1994*b*, Bollinger and Gavin 1992, Vickery et al 1994, Bollinger 1995), however, Johnson and Ingl (2001) suggest regional variability in area sensitivity. Additional studies suggest that patch shape may be an important factor regulating Grasshopper Sparrow density because this species generally avoids habitats with high perimeter-area ratios (Wiens 1969, Johnson and Temple 1990, Bock et al. 1999, Helzer and Jelinski 2001).

Mowing is one cost effective method available to resource managers interested in reducing vegetation height and controlling the encroachment of woody vegetation. Mowing practices should be deferred until after the breeding season (mid-September). Herkert (1994*b*) found that Grasshopper Sparrow densities in Illinois were twice as high on sites that were mowed 1-4 months prior to arrival on breeding grounds. Warner (1992) found that Illinois roadsides that were not mowed until August supported nest densities that were much greater than

roadsides that were mowed repeatedly throughout the breeding season. Delany et al. (1985) in Florida and Whitmore (1981) in West Virginia suggested that prescribed burning at regular intervals (2-3 years) in the winter may create suitable nesting habitat for Grasshopper Sparrows. Late winter burns on MTMVF complexes would reduce excess litter and shrub densities and also create open areas on these sites. Grazing activities on mountaintop mines are rare due to the location and isolation of these sites. However, grazing practices have occurred on at least one of our study sites. I recommend that if grazing practices are used to manage MTMVF grasslands, they be deferred until after the nesting season to minimize overgrazing, erosion, and potential trampling of nests (Whitmore 1981, Rodenhouse and Best 1983, Best et al. 1995, Rohrbaugh et al. 1999).

Reclamation practices on MTMVF complexes must comply with the 1977 Surface Mining Control and Reclamation Act. Reclamation of mined areas should include planting a mixture of bunch grasses and forb species if Grasshopper Sparrow breeding is to be encouraged (Whitmore 1981). Grasshopper Sparrows examined in this study consistently chose nest sites in areas with a high abundance of bunch grasses (e.g. broomsedge, orchard grass, tall fescue) as opposed to areas containing high amounts of turf grasses and dense vegetation (e.g. sericea, multiflora rose).

In summary, although MTMVF complexes in southern West Virginia result in the loss of contiguous forest habitat important to forest interior bird species (Wood et al. 2001, Weakland and Wood 2003, Bosworth 2003), they do provide breeding habitat for grassland bird species, especially Grasshopper Sparrows. Grasshopper Sparrows on these sites prefer grassland areas with some vertical structure and adequate bare ground cover. However, as reclamation age increases, densities and survival rates decrease suggesting that suitable habitat on these mine complexes is temporary. Management will have to be implemented to improve existing grassland areas and to maintain early successional characteristics.

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Table 1. Mist net effort, abundance, and the distribution of Grasshopper Sparrows captured and banded on three mountaintop mining / valley fill complexes and a non-mined reference area in southern West Virginia, 2001-2002.

Sites / Plots	Males	Females	Juveniles	Total Captures	Net Hours	Cap/Net Hour	Area (ha)	Individuals /ha
Cannelton	60	15	13	88	491.25	0.18	128.2	0.69
CLF-2001	21	7	2	30	124.00	0.24	36.1	0.83
CLF-2002	13	0	0	13	125.00	0.10	36.1	0.36
CCC-2001	11	7	3	21	72.25	0.29	28.0	0.75
CCC-2002	15	1	8	24	170.00	0.14	28.0	0.86
Dal-Tex	81	18	33	132	666.8	0.20	170.2	0.78
DRH-2001	29	7	2	38	217.63	0.26	45.1	0.84
DRH-2002	11	3	4	18	57.17	0.11	45.1	0.40
DSF-2001	27	4	14	45	85.00	0.26	40.0	1.13
DSF-2002	14	4	13	31	207.00	0.15	40.0	0.78
Hobet 21	128	32	28	188	824.35	0.23	204.4	0.92
HAF-2001	30	3	6	39	210.25	0.19	51.3	0.76
HAF-2002	33	7	8	48	230.52	0.21	51.3	0.94
HST-2001	22	6	2	30	76.50	0.33	50.9	0.59
HST-2002	43	16	12	71	307.08	0.23	50.9	1.39
Overall	271	65	79	415	2031.10	0.20	502.8	0.83
2001	140	33	29	202	785.63	0.26	251.4	0.74
2002	131	32	50	213	1245.47	0.17	251.4	0.78
MRWMA 2002	2	0	5	7	48.70	0.14	23.0	0.30

Table 2. Mist net effort, abundance, and distribution by age of reclamation of Grasshopper Sparrows captured and banded on three mountaintop mining / valley fill complexes in southern West Virginia, 2001-2002.

Age of Reclamation	Males	Females	Juveniles	Total Captures	Net Hours	Cap/Net Hour	Area (ha)	Individuals /ha
0-10 years								
Overall	106	30	41	177	675.6	0.26	181.8	0.97
2001	49	10	16	75	161.5	0.46	90.9	0.83
2002	57	20	25	102	514.1	0.20	90.9	1.12
11-20 years								
Overall	129	28	31	188	957.8	0.20	248.8	0.76
2001	70	17	11	98	500.1	0.20	124.4	0.79
2002	59	11	20	90	457.7	0.20	124.4	0.72
20+ years								
Overall	34	2	2	38	249.0	0.17	72.2	0.53
2001	21	2	2	25	124.0	0.24	36.1	0.7
2002	13	0	0	13	125.0	0.10	36.1	0.36

Table 3. List of non-target avian species captured by mist nets on three mountaintop mining / valley fill complexes in southern West Virginia, 2001-2002.

Species	Males	Females	Juveniles	Total Captured
Northern rough-winged swallow (*Stelgidopteryx serripennis*)	3	0	0	3
Indigo bunting (*Passerina cyanea*)	5	4	0	9
Field sparrow (*Spizella pusilla*)	9	2	0	11
Vesper sparrow (*Pooecetes gramineus*)	1	0	0	1
Savannah sparrow (*Passerculus sandwichensis*)	2	1	1	4
Nelson's sharp-tailed sparrow (*Ammodramus nelsoni*)	1	0	0	1
Eastern meadowlark (*Sturnella magna*)	5	1	2	8
American goldfinch (*Carduelis tristis*)	11	6	0	17
Willow flycatcher (*Empidonax traillii*)	1	0	0	1
Ruby-throated hummingbird (*Archilochus alexandri*)	2	0	0	2
Yellow Warbler (*Dendroica petechia*)	3	1	0	4
Prairie Warbler (*Dendroica discolor*)	2	0	0	2
Wild turkey (*Meleagris gallopavo*)	0	0	3	3
Yellow-billed cuckoo (*Coccyzus americanus*)	1	0	0	1
Orchard oriole (*Icterus spurius*)	2	1	1	4
Cedar waxwing (*Bombycilla cedrorum*)	1	0	0	1
Killdeer (*Charadrius vociferus*)	0	0	3	3
Brown-headed cowbird (*Molothrus ater*)	0	1	0	1

Table 4. Systematic nest search effort and Grasshopper Sparrow nest density on three mountaintop mining / valley fill complexes and a non-mined reference area in southern West Virginia, 2001-2002. DO and HO nests were located off of the study plots and therefore were not located by systematic nest search and are not included in the mine and overall estimates.

Site / Plots	Nest Search Effort (h)	Nests Found	Nests/hour	Area (ha)	Nests/ha
Cannelton	169.15	20	0.12	128.2	0.16
CLF-2001	72.57	4	0.06	36.1	0.11
CLF-2002	11.50	3	0.26	36.1	0.08
CCC-2001	44.33	3	0.07	28.0	0.11
CCC-2002	40.75	10	0.25	28.0	0.36
Dal-Tex	133.70	25	0.20	170.2	0.15
DSF-2001	48.91	10	0.20	40.0	0.25
DSF-2002	39.10	4	0.10	40.0	0.10
DRH-2001	26.00	5	0.19	45.1	0.11
DRH-2002	19.34	6	0.31	45.1	0.13
DOP-2001	0.33	2	6.06	-	-
Hobet 21	244.49	26	0.11	204.4	0.13
HAF-2001	108.50	7	0.65	51.3	0.14
HAF-2002	31.50	7	0.22	51.3	0.14
HST-2001	69.24	4	0.06	50.9	0.08
HST-2002	25.75	8	0.31	50.9	0.16
HOP-2001	2.00	2	0.50	-	-
HOP-2002	7.50	0	0	-	-
Overall	563.71	71	0.13	502.8	0.14
2001	372.14	33	0.09	251.4	0.13
2002	191.57	38	0.20	251.4	0.15
MRWMA-2002	13.50	0	0	23.0	0

Table 5. Grasshopper Sparrow nest density by age of reclamation on three mountaintop mining / valley fill complexes in southern West Virginia, 2001-2002. Four nests found off of study plots were not included in density estimates.

		Number of Nests		
Reclamation Age	Area (ha)	2001	2002	Nests/ha
0-10 years	181.8	14	13	0.15
11-20 years	248.8	15	24	0.16
20+ years	72.2	4	1	0.07
Overall	502.8	33	38	0.14

Table 6. Number of Grasshopper Sparrow nests on three mountaintop mining / valley fill complexes in southern West Virginia, 2001-2002 using each vegetation species as the primary nest construction substrate.

	Hobet 21		Dal-Tex		Cannelton		
Species	HAF	HSF	DRH	DSF	CLF	CCC	Overall
Broomsedge (*Andropogon virginianus*)	6	2	8	0	4	3	23
Tall fescue (*Festuca arundinacea*)	5	5	1	10	0	4	25
Orchard grass (*Dactylis glomerata*)	3	2	0	4	0	4	13
Perennial ryegrass (*Lolium perenne*)	0	2	0	0	1	3	6
Birdsfoot-trefoil (*Lotus corniculatus*)	1	0	0	0	0	2	3
Alfalfa (*Medicago sativa*)	0	0	0	1	0	0	1
Sericea (*Lespedesa cuneata*)	0	0	2	0	0	0	2
Mammoth red clover (*Trifolium pratense*)	0	0	1	0	0	0	1
Golden rod (*Solidago virgauria*)	0	0	1	0	0	0	1

Table 7. Grasshopper Sparrow nest clump vegetation composition (N=75) on three mountaintop mining / valley fill complexes in southern West Virginia, 2001-2002. Table entries are the number of nests constructed in these species.

Nest Substrate	Primary[a]	Secondary[b]	Tertiary[c]	Overall
Broomsedge (*Andropogon virginianus*)	23	1	2	26
Tall fescue (*Festuca arundinacea*)	25	15	3	43
Orchard grass (*Dactylis glomerata*)	13	3	3	19
Perennial ryegrass (*Lolium perenne*)	6	1	0	7
Birdsfoot-trefoil (*Lotus corniculatus*)	3	37	10	50
Alfalfa (*Medicago sativa*)	1	0	0	1
Sericea (*Lespedesa cuneata*)	2	1	2	5
Mammoth red clover (*Trifolium pratense*)	1	7	4	12
Golden rod (*Solidago virgauria*)	1	0	0	1
Wild Timothy (*Muhlenbergia glomerata*)	0	1	1	2
Moss spp.	0	1	0	1
Aster spp.	0	1	3	4

[a] nest were constructed directly in primary substrates
[b] secondary substrates were the second most dominant species in the nest clump
[c] tertiary substrates were the third most dominant species in the nest clump

Table 8. Mean and standard error (SE) of clutch size and the number of offspring fledged for Grasshopper Sparrow nests characterized by site and year in southern West Virginia in 2001 and 2002.

		Clutch Size		Number Fledged	
Sites / Plots	N	Mean	SE	Mean	SE
Cannelton					
CLF-2001	4	3.25	0.75	1.25	1.25
CLF-2002	1	5.00	0.00	5.00	0.00
CCC-2001	3	4.00	0.00	0.00	0.00
CCC-2002	13	4.15	0.15	1.70	0.56
Dal-Tex					
DOP-2001	2	3.50	0.50	3.50	0.50
DRH-2001	5	3.40	0.60	0.80	0.80
DRH-2002	6	3.83	0.31	1.67	0.80
DSF-2001	10	3.80	0.33	2.60	0.64
DSF-2002	5	3.60	0.24	2.80	0.73
Hobet 21					
HAF-2001	8	3.88	0.23	1.40	0.68
HAF-2002	5	3.60	0.24	1.60	0.98
HST-2001	3	3.67	0.67	2.67	1.45
HST-2002	8	3.37	0.18	1.50	0.60
HOP-2001	2	4.50	0.50	2.00	2.00
Overall	75	3.77	0.09	1.81	0.22
2001	37	3.73	0.16	1.76	0.33
2002	38	3.82	0.11	1.87	0.30

Table 9. Mean and standard error (SE) clutch size and the number of offspring fledged from monitored nests characterized by reclamation age (N=94) on three mountaintop mining / valley fill complexes in southern West Virginia, 2001-2002.

		Clutch Size			Number Fledged		
Reclamation Age	N	Mean	SE	Range	Mean	SE	Range
0-10 years	26	3.62	0.16	2-5	2.3	0.36	0-5
11-20 years	63	4.03	0.10	1-5	1.7	0.24	0-5
20+ years	5	3.60	0.70	2-5	2.0	1.22	0-5

Table 10. Mean and standard error (SE) clutch size and the number of offspring fledged from monitored nests characterized by month of initiation (N=94) on three mountaintop mining / valley fill complexes in southern West Virginia, 2001-2002.

		Clutch Size			Number Fledged		
Month	N	Mean	SE	Range	Mean	SE	Range
May	17	4.6	0.14	4-5	1.76	0.50	0-5
June	40	4.1	0.10	3-5	2.23	0.33	0-5
July	31	3.4	0.15	1-5	1.25	0.29	0-5
August	5	3.2	0.20	3-4	3.20	0.20	3-4
Overall	94	3.9	0.09	1-5	1.86	0.20	0-5

Table 11. Nest survival estimates for 94 Grasshopper Sparrows nests on mountaintop mining / valley fill complexes in southern West Virginia from 1999-2002.

Mine	Year	N	Observation Days	Incubation Survival	Brooding Survival	Total Survival
Daltex	1999	1	4.5	0.06	0.00	0.00
	2000	7	68.0	0.48	1.00	0.48
	2001	16	166.0	0.71	0.60	0.43
	2002	11	116.0	0.75	0.70	0.53
	99-02	36	375.5	0.66	0.72	0.48
Hobet 21	1999	3	32.0	0.49	0.55	0.27
	2000	5	58.5	1.00	1.00	1.00
	2001	13	111.0	0.31	0.74	0.23
	2002	13	123.0	0.35	0.50	0.18
	99-02	34	324.5	0.41	0.68	0.28
Cannelton	1999	0	0.0	0.00	0.00	0.00
	2000	3	9.0	0.03	1.00	0.03
	2001	7	68.0	0.26	0.63	0.16
	2002	14	190.0	0.77	0.63	0.49
	99-02	24	267.0	0.47	0.63	0.30
Overall	99-02	94	967.0	0.50	0.69	0.35

Table 12. Nest survival estimates for 94 Grasshopper Sparrows nests on mountaintop mine / valley fill complexes classified by post reclamation age. Numbers in parenthesis are survival estimates excluding nests that failed due to human disturbance.

Age of Reclamation	N	Observation Days	Incubation Survival	Brooding Survival	Total Survival
0-10 years	33	355 (333.5)	0.54 (0.56)	0.79 (0.82)	0.43 (0.46)
11-20 years	50	525 (516.5)	0.55 (0.59)	0.62 (0.63)	0.34 (0.38)
20+ years	11	77 (68)	0.16 (0.16)	0.82 (1.00)	0.13 (0.16)

Table 13. Common and scientific names for potential nest predators observed on grassland areas of mountaintop mine / valley fill complexes in southern West Virginia from 2001-2002.

Common Name	Scientific Name	Sighting
Black Rat Snake	*Elaphe obsoleta obsoleta*	Common
Northern Black Racer	*Coluber constrictor constrictor*	Common
Northern Harrier	*Circus cyaneus*	Common
Short-eared Owl[a]	*Asio flammeus*	Rare
American Kestrel	*Falco sparverius*	Common
Red-tailed Hawk	*Buteo jamaicensis*	Common
White-tailed Kite[b]	*Elanus leucurus*	Rare
Cooper's Hawk	*Accipiter cooperii*	Rare
American Crow	*Corvus brachyrhynchos*	Common
Blue Jay	*Cyanocitta cristata*	Rare
White-footed Mouse[c]	*Peromyscus leucopus*	Common
Deer Mouse[c]	*Peromyscus maniculatus*	Common
Red Fox	*Vulpes vulpes*	Rare
Gray Fox	*Urocyon cinereoargenteus*	Rare
Bobcat	*Lynx rufus*	Rare
Domestic Dog	*Canis familiaris*	Common
Domestic Cat	*Felis domestica*	Rare

[a]Ammer and Wood 2003
[b]Wood et al.2002
[c]Chamblin 2002

Table 14. Mean and standard error (SE) of nest and habitat variables surrounding successful (n=37) and unsuccessful (n=38) nests of Grasshopper Sparrows on mountaintop mine / valley fill complexes in 2001-2002. Analysis of variance (ANOVA) was used to compare habitat variables (α=0.10).

	Successful		Unsuccessful		ANOVA	
Variable	Mean	SE	Mean	SE	F	P
Slope Aspect	1.14	0.12	1.31	0.11	0.11	0.74
Slope	1.79	0.10	8.30	3.00	1.24	0.29
Overhead Cover (%)	84.54	3.62	83.78	3.39	0.84	0.38
Side Cover (%)	89.53	1.64	89.12	1.19	0.14	0.72
Distance to Minor Edge (m)	16.42	3.84	23.08	5.06	0.24	0.63
Percent Green	53.28	5.75	51.41	6.10	0.03	0.88
Ground Cover (%)						
Grass	50.00	0.49	52.05	0.45	0.02	0.90
Forb	34.00	0.47	35.25	0.48	0.35	0.57
Litter	4.00	0.20	1.95	0.11	0.64	0.44
Bare ground	10.4	0.40	9.30	0.38	0.06	0.80
Moss	1.35	0.08	1.20	0.09	0.14	0.71
Robel Pole Index (dm)						
Nest	2.40	0.19	3.14	0.30	0.27	0.62
1m	2.43	0.23	3.22	0.32	0.32	0.58
3m	2.58	0.33	3.12	0.31	1.09	0.32
5m	2.55	0.29	3.00	0.29	0.03	0.86
Grass Height (dm)						
1m	3.34	0.25	3.40	0.17	0.02	0.89
3m	4.21	0.72	4.68	1.12	0.05	0.83
5m	3.36	0.21	3.57	0.22	0.02	0.73
10m	4.22	0.25	4.11	0.18	0.01	0.93
Litter depth (cm)						
1m	0.25	0.06	0.24	0.04	0.02	0.88
3m	0.24	0.05	0.29	0.05	0.00	0.95
5m	0.26	0.06	0.22	0.03	0.00	0.99
10m	0.21	0.04	0.26	0.04	0.31	0.59
% Woody Cover on Plot	2.77	0.55	4.40	0.85	1.77	0.57
Tree Dist. from Nest (m)	6.88	0.61	6.74	0.70	1.61	0.33
Dominant Tree Height (m)	0.74	0.08	1.03	0.18	0.75	0.48
Dominant Tree Width (m)	0.48	0.07	0.77	0.20	1.00	0.42
Shrub Dist. from Nest (m)	6.88	0.60	7.40	0.51	0.39	0.57
Dominant Shrub Height (m)	1.33	0.14	1.37	0.15	0.63	0.47
Dominant Shrub Width (m)	1.04	0.11	1.35	0.17	6.63	0.06
Nest substrate height (veg)(cm)	3.69	0.16	3.87	0.20	0.08	0.78
Nest substrate height (repro)(cm)	7.15	0.38	6.55	0.34	6.58	0.04
Nest Clump Area (cm^2)	1,454.35	139.09	1,672.74	226.19	1.09	0.32
Distance to foliage edge (cm)	16.95	1.77	19.82	1.41	3.17	0.11
Nest depth (cm)	6.06	0.17	6.04	0.18	0.01	0.94
Nest rim width (cm)	1.86	0.07	1.81	0.06	0.14	0.72
Nest rim height (cm)	1.62	0.17	1.46	0.20	0.06	0.81

Table 15. Mean number and standard error (SE) of vegetation species relative abundance found in the vicinity of successful and unsuccessful nests on MTRVF areas in 2001-2002. Analysis of variance (ANOVA) was used to compare species abundances (α=0.10). Dashed lines (--) indicate the inability to perform statistical analyses due to low occurrence of those variables on sample plots.

	Successful		Unsuccessful		ANOVA	
Variable	Mean	± SE	Mean	± SE	*F*	*P*
Broomsedge (*Andropogon virginianus*)	1.22	0.36	1.80	0.39	1.52	0.25
Orchard grass (*Dactylis glomerata*)	2.65	0.39	1.84	0.36	0.13	0.73
Tall fescue (*Festuca arundinacea*)	5.0	0.53	5.24	0.58	0.03	0.86
Perennial ryegrass (*Lolium perenne*)	0.70	0.23	1.13	0.25	1.86	0.20
Wild Timothy (*Muhlenbergia glomerata*)	0.11	0.05	0.11	0.06	--	--
Hairgrass (*Agrostis scabra*)	0.14	0.09	0.00	0.00	--	--
Smooth Brome (*Bromus inermis*)	0.10	0.04	0.00	0.00	--	--
Birdsfoot-trefoil (*Lotus corniculatus*)	4.32	0.37	4.0	0.43	0.22	0.65
Mammoth red clover (*Trifolium pratense*)	0.57	0.15	0.45	0.14	0.54	0.48
Sericea (*Lespedesa cuneata*)	0.43	0.15	1.18	0.33	1.87	0.21
Golden rod (*Solidago virgauria*)	0.05	0.04	0.00	0.00	--	--
Alfalfa (*Medicago sativa*)	0.38	0.14	0.21	0.09	0.04	0.84
Carex spp.	0.11	0.11	0.00	0.00	--	--
Wild lettuce (*Lactuca canadensis*)	0.03	0.03	0.00	0.00	--	--
Aster spp.	0.59	0.23	0.42	0.13	0.21	0.66
Sweet clover (*Melilotus alba*)	0.24	0.10	0.29	0.12	0.00	0.95
Cattail *(Typha latifolia)*	0.05	0.05	0.00	0.00	--	--
Common plantain *(Plantago major*)	0.00	0.00	0.03	0.03	--	--
Horseweed (*Erigeron canadensis*)	0.19	0.13	0.18	0.14	--	--
Crown vetch (*Coronilla varia*)	0.11	0.08	0.11	0.08	--	--

Table 16. Mean and standard error (SE) of habitat variables measured at nests (N=75) and fixed habitat plots (N=56) on mountaintop mine / valley fill complexes in 2001-2002. Analysis of variance (ANOVA) was used to compare habitat variables (α=0.10).

	Nests		Habitat Plots		ANOVA	
Variable	Mean	SE	Mean	SE	F	P
Slope Aspect	1.23	0.08	1.15	0.06	1.18	0.31
Slope	1.78	0.01	1.78	0.01	0.49	0.50
Dist. to Minor Edge (m)	19.79	3.19	40.67	6.98	0.63	0.43
Percent Green	52.34	4.17	59.53	3.70	19.66	0.002
Ground Cover (%)						
Grass	50.90	0.33	56.05	0.38	0.04	0.84
Forb	34.85	0.33	37.10	0.35	0.28	0.61
Shrub	0.00	0.00	0.25	0.02	0.56	0.46
Litter	20.85	0.11	18.50	0.11	4.30	0.07
Bare ground	14.00	0.18	7.75	0.09	5.67	0.04
Moss	1.30	0.06	0.80	0.05	0.00	0.99
Robel Pole Index (dm)						
Plot center	2.77	0.18	1.55	0.07	15.27	0.004
1m	2.82	0.20	2.07	0.08	2.81	0.13
3m	2.86	0.23	2.00	0.08	1.88	0.20
5m	2.76	0.20	1.97	0.09	1.99	0.19
Grass Height (dm)						
1m	3.37	0.15	4.94	1.06	20.46	0.001
3m	4.48	0.66	3.93	0.11	6.83	0.03
5m	3.46	0.15	4.13	0.13	2.42	0.15
10m	4.17	0.15	4.36	0.15	0.08	0.78
Litter depth (cm)						
1m	0.25	0.03	0.17	0.03	3.47	0.09
3m	0.27	0.03	0.18	0.03	1.12	0.32
5m	0.24	0.04	0.18	0.02	4.27	0.07
10m	0.23	0.03	0.17	0.02	0.34	0.57
% Woody Cover on Plot	3.54	0.90	7.10	1.51	0.27	0.62
Tree Stem Density	3.70	1.30	3.42	2.10	2.40	0.16
Tree Dist. from Nest (m)	7.40	1.29	6.86	0.64	0.00	0.95
Dominant Tree Height (m)	1.16	0.29	0.92	0.19	0.13	0.73
Dominant Tree Width (m)	0.91	0.33	0.66	0.21	2.35	0.22
Shrub Stem Density	4.50	0.54	4.20	1.00	9.00	0.01
Shrub Dist. from Nest (m)	7.03	0.60	7.34	0.41	0.37	0.60
Dominant Shrub Height (m)	1.38	1.65	1.41	0.09	0.39	0.55
Dominant Shrub Width (m)	1.26	0.19	1.18	0.11	1.92	0.21

Table 17. Mean number and standard error (SE) for vegetation species relative abundance at nests (N=75) and fixed habitat plots (N=56) on mountaintop mine / valley fill complexes in 2001-2002. Analysis of variance (ANOVA) was used to compare habitat variables (α=0.10). Dashed lines (--) indicate the inability to perform statistical analyses due to very low occurrence of those variables on sample plots.

	Nests		Habitat Plots		ANOVA	
Variable	Mean	SE	Mean	SE	*F*	*P*
Broomsedge (*Andropogon virginianus*)	1.51	0.27	2.0	0.28	3.45	0.09
Orchard grass (*Dactylis glomerata*)	2.24	0.27	2.30	0.30	0.66	0.44
Tall fescue (*Festuca arundinacea*)	5.12	0.38	5.92	0.41	0.54	0.48
Perennial ryegrass (*Lolium perenne*)	0.92	0.17	0.84	0.21	0.21	0.66
Wild Timothy (*Muhlenbergia glomerata*)	0.11	0.04	0.13	0.04	0.38	0.55
Hairgrass (*Agrostis scabra*)	0.07	0.04	0.00	0.00	--	--
Smooth Brome (*Bromus inermis*)	0.02	0.01	0.05	0.02	0.00	0.97
Birdsfoot-trefoil (*Lotus corniculatus*)	4.16	0.03	3.81	0.27	0.55	0.47
Mammoth red clover (*Trifolium pratense*)	0.51	0.10	0.54	0.09	0.81	0.39
Sericea (*Lespedesa cuneata*)	0.81	0.19	1.71	0.32	5.00	0.05
Golden rod (*Solidago virgauria*)	0.27	0.02	0.11	0.05	4.43	0.06
Alfalfa (*Medicago sativa*)	0.29	0.08	0.42	0.13	0.03	0.86
Carex spp.	0.05	0.05	0.00	0.00	--	--
Wild lettuce (*Lactuca canadensis*)	0.01	0.01	0.02	0.01	0.22	0.65
Aster spp.	0.51	0.13	0.33	0.06	0.92	0.36
Sweet clover (*Melilotus alba*)	0.27	0.08	0.15	0.05	7.26	0.02
Cattail *(Typha latifolia)*	0.03	0.03	0.00	0.00	--	--
Common plantain *(Plantago major*)	0.00	0.00	0.04	0.03	--	--
Horseweed (*Erigeron canadensis*)	0.19	0.09	0.01	0.01	--	--
Crown vetch (*Coronilla varia*)	0.11	0.06	0.26	0.14	--	--
Bicolor lespedeza (*Lespedeza bicolor*)	0.00	0.00	0.02	0.02	--	--
Autumn olive (*Eleaganus umbellata*)	0.00	0.00	0.02	0.01	--	--
Multiflora rose (*Rosa multiflora*)	0.00	0.00	0.01	0.01	--	--
Sedge spp.	0.00	0.00	0.02	0.02	--	--
Rubus spp.	0.00	0.00	0.01	0.01	--	--

Table 18. Mean and standard error (SE) of nest variables classified by reclamation age. Analysis of variance (ANOVA) was used to compare habitat variables on mountaintop mine / valley fill complexes in 2001-2002, (α=0.10). In a row, variables with the same letter did not differ with the Waller-Duncan K-ratio t-tests (W-D).

	0-10 years			11-20 years			20+ years				
Variable	Mean	SE	W-D	Mean	SE	W-D	Mean	SE	W-D	*F*	*P*
Slope Aspect	1.29	0.13	A	1.14	0.12	A	1.65	0.15	A	1.56	0.30
Slope	1.77	0.02	A	1.78	0.02	A	1.80	0.04	A	0.46	0.66
Overhead Cover (%)	84.37	4.76	A	84.53	2.95	A	80.00	9.40	A	0.19	0.83
Side Cover (%)	88.66	1.92	A	89.88	1.24	A	87.50	2.80	A	0.04	0.96
Dist. to Minor Edge (m)	13.80	3.10	A	20.64	4.90	A	45.00	11.20	A	0.52	0.62
Percent Green	48.50	6.84	A	57.23	5.62	A	31.20	12.21	A	1.50	0.31
Ground Cover (%)											
Grass	53.00	0.44	A	49.80	0.44	A	60.00	2.12	A	1.17	0.38
Forb	30.00	0.45	A	38.00	0.44	A	36.00	2.03	A	1.32	0.35
Litter	1.65	0.18	A	3.70	0.17	A	0.00	0.00	A	1.36	0.34
Bare ground	13.90	0.51	A	7.80	0.32	A	4.00	0.57	A	0.98	0.44
Moss	1.10	0.08	A	1.55	0.09	A	0.00	0.00	A	0.62	0.58
Robel Pole Index (dm)											
Nest	2.29	0.18	A	2.98	0.27	A	3.60	0.85	A	0.62	0.57
1m	2.18	0.19	A	3.10	0.31	A	4.00	0.70	A	0.44	0.66
3m	2.16	0.18	A	3.20	0.35	A	3.93	1.24	A	1.16	0.38
5m	2.17	0.23	A	3.10	0.31	A	3.35	0.76	A	1.48	0.31
Grass Height (dm)											
1m	3.30	0.29	A	3.41	0.19	A	3.42	0.47	A	0.23	0.80
3m	3.40	0.27	A	4.65	0.99	A	8.40	5.04	B	10.10	0.02
5m	3.31	0.24	A	3.62	0.22	A	3.00	0.40	A	0.12	0.90
10m	3.93	0.32	A	4.35	0.20	A	3.86	0.17	A	0.21	0.82
Litter depth (cm)											
1m	0.21	0.05	A	0.26	0.10	A	0.33	0.12	A	0.41	0.68
3m	0.24	0.06	A	0.28	0.04	A	0.29	0.04	A	0.06	0.95
5m	0.17	0.03	A	0.27	0.10	A	0.37	0.10	A	0.55	0.45
10m	0.22	0.05	A	0.24	0.04	A	0.26	0.09	A	0.06	0.94
% Woody Cover on Plot	2.05	0.54	A	4.71	1.25	A	5.75	0.25	A	0.51	0.63
Tree Stem Density	3.05	0.56	A	5.07	3.56	A	1.25	1.25	A	1.88	0.21
Shrub Stem Density	2.34	0.63	A	2.53	1.66	A	7.25	0.75	A	0.16	0.86
Shrub Dist. from Nest (m)	6.59	1.01	A	7.77	0.61	A	9.48	1.55	A	1.70	0.27
Dom. Shrub Height (m)	1.33	0.22	A	1.39	0.18	A	1.36	0.02	A	0.23	0.80

Table 18. Cont.

	0-10 years			11-20 years			20+ years				
Variable	Mean	SE	W-D	Mean	SE	W-D	Mean	SE	W-D	*F*	*P*
Dom. Shrub Width (m)	1.12	0.24	A	1.29	0.21	A	0.75	0.10	A	0.32	0.74
Nest Substrate height (veg)(cm)	3.98	0.21	A	3.62	0.17	A	4.00	0.59	A	0.11	0.90
Nest Substrate height (repro)(cm)	7.64	0.37	A	6.22	0.35	A	7.30	0.77	A	0.85	0.49
Nest Clump Area (cm^2)	1194.91	78.99	A	1779.95	215.83	A	1736.40	469.61	A	0.64	0.56
Distance to foliage edge (cm)	18.19	1.82	A	18.61	1.52	A	17.60	5.63	A	0.13	0.88

Table 19. Mean number and standard error (SE) of vegetation species relative abundance at nests characterized by post reclamation age. Analysis of variance (ANOVA) was used to compare habitat variables (α=0.10). In a row, variables with the same letter did not differ with the Waller-Duncan K-ratio t-tests (W-D) at α=0.10.

	0-10 years			11-20 years			20+ years			ANOVA	
Variable	Mean	SE	W-D	Mean	SE	W-D	Mean	SE	W-D	*F*	*P*
Broomsedge (*Andropogon virginianus*)	0.63	0.25	A	2.02	0.41	A	1.80	0.73	A	0.04	0.96
Orchard grass (*Dactylis glomerata*)	2.74	0.47	A	2.10	0.35	A	1.00	0.55	A	0.61	0.58
Tall fescue (*Festuca arundinacea*)	6.20	0.50	A	4.12	0.53	A	8.00	0.84	B	8.16	0.03
Perennial ryegrass (*Lolium perenne*)	0.85	0.31	A	0.93	0.21	A	1.20	0.80	A	0.19	0.83
Birdsfoot-trefoil (*Lotus corniculatus*)	3.89	0.37	A	4.47	0.41	A	3.00	1.14	A	0.05	0.95
Mammoth red clover (*Trifolium pratense*)	0.85	0.21	A	0.28	0.09	A	0.60	0.40	A	1.47	0.31
Sericea (*Lespedesa cuneata*)	0.11	0.08	A	1.09	0.27	A	2.20	1.20	A	1.28	0.36
Alfalfa (*Medicago sativa*)	0.78	0.19	A	0.02	0.02	B	0.00	0.00	B	28.02	0.002
Aster spp.	0.07	0.05	A	0.84	0.21	A	0.00	0.00	A	2.06	0.22
Sweet clover (*Melilotus alba*)	0.37	0.14	A	0.09	0.04	B	1.20	0.73	C	13.94	0.009

Table 20. Mean and standard error (SE) for habitat variables measured at nests (N=75) and fixed habitat plots (N=56) on mountaintop mine / valley fill complexes in 2001-2002. Analysis of variance (ANOVA) was used to compare habitat variables among reclamation age classes (α=0.10).

	Nests						Habitat Plots							
	0-10 years		11-20 years		20+ years		0-10 years		11-20 years		20+ years		ANOVA	
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*F*	*P*
Slope Aspect	1.30	0.13	1.16	0.12	1.65	0.15	1.30	0.12	1.10	0.09	1.20	0.15	2.55	0.13
Slope	1.77	0.02	1.77	0.02	1.79	0.04	1.80	0.02	1.77	0.01	1.76	0.02	0.10	0.90
Percent Green	48.50	6.84	61.33	5.82	31.2	12.21	52.94	6.12	58.70	5.70	57.75	10.02	9.82	0.01
Ground Cover (%)														
Grass	53.00	0.44	49.20	0.46	60.00	2.12	52.00	0.57	55.75	0.53	59.05	1.25	1.11	0.37
Forb	30.00	0.45	36.70	0.44	36.00	2.03	37.15	0.55	38.75	0.51	36.55	1.14	0.24	0.79
Litter	1.65	0.13	4.10	0.19	0.00	0.00	3.90	0.23	3.25	0.16	1.55	0.20	3.83	0.06
Bare ground	13.90	0.51	7.70	0.34	4.00	0.60	4.55	0.18	1.45	0.11	2.80	0.33	3.14	0.09
Moss	1.10	0.08	1.60	0.10	0.00	0.00	1.55	0.09	0.65	0.07	0.00	0.00	1.05	0.39
Robel Pole Index (dm)														
Plot center	2.91	0.12	2.96	0.30	3.58	0.85	1.30	0.12	1.64	0.09	1.74	0.17	1.98	0.19
1m	2.18	0.19	3.11	0.34	4.00	0.70	1.70	0.10	2.30	0.13	2.18	0.14	3.41	0.08
3m	2.16	0.18	3.14	0.40	3.93	1.24	1.63	0.11	2.11	0.11	2.10	0.24	2.85	0.11
5m	2.17	0.23	2.97	0.32	3.40	0.76	1.50	0.10	2.13	0.13	2.15	0.22	1.85	0.21
Grass Height (dm)														
1m	3.29	0.29	3.51	0.20	3.42	0.47	3.25	0.18	4.20	0.16	10.61	6.83	12.64	0.002
3m	3.40	0.27	4.88	1.10	8.40	5.05	3.73	0.23	4.04	0.16	3.70	0.19	4.39	0.05
5m	3.31	0.24	3.65	0.23	3.00	0.40	3.58	0.26	4.40	0.16	4.12	0.28	0.72	0.51
10m	3.93	0.32	4.42	0.18	3.86	0.17	3.80	0.24	4.54	0.16	3.95	0.20	1.74	0.23
Litter depth (cm)														
1m	0.21	0.05	0.28	0.06	0.33	0.12	0.11	0.02	0.17	0.02	0.27	0.07	3.37	0.08
3m	0.24	0.06	0.28	0.05	0.30	0.04	0.13	0.02	0.17	0.02	0.35	0.14	1.99	0.19
5m	0.17	0.03	0.27	0.06	0.37	0.01	0.14	0.02	0.17	0.02	0.32	0.01	6.17	0.02
10m	0.22	0.05	0.25	0.05	0.26	0.09	0.12	0.02	0.16	0.02	0.31	0.09	1.43	0.29
% Woody Cover on Plot	2.13	0.40	4.92	0.91	4.33	2.84	5.78	1.68	4.56	0.93	4.50	1.30	6.98	0.02
Tree Stem Density	0.80	0.58	4.14	3.52	0.00	0.00	5.29	3.43	6.00	3.58	2.50	2.50	1.88	0.21
Shrub Stem Density	2.37	0.41	2.89	0.99	5.67	3.28	2.62	0.56	7.47	2.15	13.2	5.39	1.55	0.26
Shrub Dist. from Nest (m)	6.41	0.68	5.57	0.46	8.93	2.10	7.99	0.49	7.76	0.44	7.53	0.88	1.71	0.25
Dom. Shrub Height (m)	1.30	0.21	1.40	0.12	1.40	0.10	1.54	0.13	1.30	0.08	0.77	0.07	5.87	0.03
Dom. Shrub Width (m)	1.08	0.14	1.35	0.16	0.69	0.12	1.58	0.15	0.94	0.07	0.99	0.07	6.22	0.03

Table 21. Mean and standard error (SE) for abundance of each vegetative species measured at nests (N=75) and fixed habitat plots (N=56) on mountaintop mine / valley fill complexes in 2001-2002. One-way analysis of variance (ANOVA) was used to compare habitat variables (α=0.10).

	Nests						Habitat Plots						ANOVA	
	0-10 years		11-20 years		20+ years		0-10 years		11-20 years		20+ years			
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*F*	P
Broomsedge (*Andropogon virginianus*)	0.63	0.25	2.23	0.44	1.80	0.73	1.06	0.37	2.04	0.40	3.56	0.97	8.04	0.01
Orchard grass (*Dactylis glomerata*)	2.74	0.47	2.03	0.37	1.00	0.55	3.13	0.71	2.10	0.40	1.90	0.40	1.71	0.23
Tall fescue (*Festuca arundinacea*)	6.19	0.50	4.10	0.57	8.00	0.84	5.40	0.67	5.60	0.64	6.00	0.81	3.53	0.07
Perennial ryegrass (*Lolium perenne*)	0.85	0.31	0.92	0.23	1.20	0.80	0.63	0.19	1.27	0.43	0.38	0.22	0.44	0.66
Wild Timothy (*Muhlenbergia glomerata*)	0.15	0.09	0.08	0.04	0.00	0.00	0.19	0.07	0.08	0.05	0.06	0.06	1.97	0.20
Smooth Brome (*Bromus inermis*)	0.04	0.04	0.03	0.03	0.00	0.00	0.03	0.03	0.04	0.03	0.00	0.00	0.27	0.77
Birdsfoot-trefoil (*Lotus corniculatus*)	3.89	0.37	4.51	0.43	3.00	1.14	4.44	0.39	4.17	0.38	2.63	0.79	0.96	0.42
Mammoth red clover (*Trifolium pratense*)	0.85	0.21	0.18	0.06	0.60	0.40	1.00	0.22	0.33	0.10	0.06	0.06	9.21	0.006
Sericea (*Lespedesa cuneata*)	0.11	0.08	1.0	0.24	2.20	1.20	0.16	0.09	2.50	0.60	3.40	1.00	25.46	0.0002
Golden rod (*Solidago virgauria*)	0.00	0.00	0.00	0.00	0.20	0.20	0.00	0.00	0.15	0.09	0.30	0.11	7.07	0.01
Alfalfa (*Medicago sativa*)	0.78	0.19	0.03	0.03	0.00	0.00	1.31	0.40	0.02	0.02	0.00	0.00	34.19	<0.0001
Aster spp.	0.07	0.05	0.82	0.22	0.00	0.00	0.19	0.09	0.30	0.08	0.63	0.18	9.89	0.005
Sweet clover (*Melilotus alba*)	0.37	0.14	0.10	0.05	1.20	0.73	0.25	0.14	0.08	0.04	0.13	0.13	5.75	0.02

Table 22. Mean and standard error (SE) for habitat variables measured in 2002 on mined (N=48) and non-mined (N=8) vegetation plots. One-way analysis of variance (ANOVA) was used to compare habitat variables between mined and non-mined plots (α=0.10). Dashed lines (--) indicate the inability to perform statistical analyses due to very low occurrence of those variables on sample plots.

	Mined		Non-mined		ANOVA	
Variable	Mean	SE	Mean	SE	*F*	*P*
Slope Aspect	1.10	0.24	1.32	0.06	0.08	0.79
Slope	1.74	0.01	1.76	0.008	0.68	0.45
Percent Green	91.01	1.07	94.53	2.70	0.02	0.90
Ground Cover (%)						
Grass	51.10	0.34	68.15	1.43	4.10	0.01
Forb	34.75	0.29	28.15	1.41	3.45	0.10
Shrub	0.10	0.02	0.00	0.00	--	--
Litter	6.05	0.13	3.15	0.18	--	--
Bare ground	6.35	0.21	0.00	0.00	1.37	0.29
Moss	1.25	0.06	0.65	0.13	0.09	0.78
Robel Pole Index (dm)						
nest	1.73	0.08	1.63	0.30	0.01	0.93
1m	1.93	0.08	2.10	0.29	0.06	0.81
3m	1.83	0.83	2.10	0.32	0.30	0.61
5m	1.73	0.09	2.60	0.50	2.83	0.15
Grass Height (dm)						
0m	3.20	0.11	2.50	0.31	0.62	0.47
1m	3.84	0.13	4.84	0.60	4.80	0.08
3m	4.86	0.60	4.53	0.35	0.75	0.43
5m	4.06	0.15	4.83	0.60	0.97	0.37
10m	4.48	0.13	6.28	1.12	19.7	<0.01
Litter depth (cm)						
1m	0.24	0.03	0.18	0.09	0.06	0.81
3m	0.23	0.03	0.11	0.03	1.52	0.27
5m	0.22	0.03	0.10	0.02	1.07	0.35
10m	0.19	0.03	0.18	0.06	0.01	0.92
Tree Dist. from Nest (m)	7.06	0.35	0.00	0.00	--	--
Tree Height (m)	0.98	0.09	0.00	0.00	--	--
Tree Width (m)	0.70	0.10	0.00	0.00	--	--
Shrub Dist. from Nest (m)	7.88	0.32	7.43	3.47	0.40	0.55
Shrub Height (m)	1.30	0.08	0.62	0.18	1.64	0.24
Shrub Width (m)	1.20	0.09	0.76	0.36	0.02	0.88
% Woody Cover on Plot	3.42	0.43	1.00	0.00	2.20	0.18

Table 23. Mean and standard error (SE) for vegetation species relative abundance at mined (N=56) and non-mined (N=8) subplots. One-way analysis of variance (ANOVA) was used to compare habitat variables (α=0.10). Dashed lines (--) indicate the inability to perform statistical analyses due to very low occurrence of those variables on sample plots.

	Mined		Non-mined		ANOVA	
Variable	Mean	SE	Mean	SE	*F*	*P*
Broomsedge (*Andropogon virginianus*)	1.41	0.27	2.40	1.07	0.31	0.60
Orchard grass (*Dactylis glomerata*)	1.50	0.21	0.75	0.37	1.36	0.30
Tall fescue (*Festuca arundinacea*)	6.40	0.41	9.90	1.51	7.44	0.04
Perennial ryegrass (*Lolium perenne*)	0.58	0.07	0.00	0.00	--	--
Birdsfoot-trefoil (*Lotus corniculatus*)	4.42	0.27	1.50	0.80	19.73	<0.01
Mammoth red clover (*Trifolium pratense*)	0.36	0.07	0.63	0.32	0.54	0.50
Sericea (*Lespedesa cuneata*)	1.07	0.20	0.00	0.00	--	--
Alfalfa (*Medicago sativa*)	0.22	0.07	0.13	0.13	0.05	0.83
Aster spp.	0.51	0.11	0.75	0.42	1.75	0.24
Sweet clover (*Melilotus alba*)	0.23	0.07	0.00	0.00	--	--
Moss spp.	0.24	0.06	0.13	0.13	0.09	0.77

Table 24. Comparison of Grasshopper Sparrow nest density and survival on the three reclaimed MTMVF complexes with those reported in previous studies.

No. Nests (years)	Nest Density[b]	Nest Survival	Location	Grassland Type[a]	Study
51 (3)	0.11/ha	0.07	West Virginia	Surface Mines	Wray (1982)
14 (3)	nr	0.11	North Dakota	WPA	Koford (1999)
38 (3)	nr	0.28	North Dakota	CRP Fields	Koford (1999)
13 (3)	nr	0.12	Minnesota	CRP Fields	Koford (1999)
12 (3)	0.25/ha	~0.25[c]	Oklahoma	TGP	Rohrbaugh et al. (1999)
23 (3)	nr	0.22	Missouri	TGP	Winter and Faaborg (1999)
38 (3)	nr	0.41	Missouri	CRP Fields	McCoy et al. (1999)
19 (2)	0.06/ha	0.36	West Virginia	MTMVF	Wood et al. (2000)
19 (2)	~0.40-0.52/ha[d]	0.46	Ohio	Surface Mines	Ingold (2002)
75 (2)	0.14/ha	0.33	West Virginia	MTMVF	This study

[a] MTMVF = mountaintop mining/valley fill; CRP = conservation reserve program; WPA = waterfowl production area; TGP = Tall Grass Prairie

[b] nr=not reported.

[c] Survival rates were presented in a figure, estimates are approximate.

[d] Nest densities were presented in a figure, estimates are approximate.

Figure 1. Locations of the three mountaintop mine / valley fill complexes and the non-mined reference area surveyed in southern West Virginia.

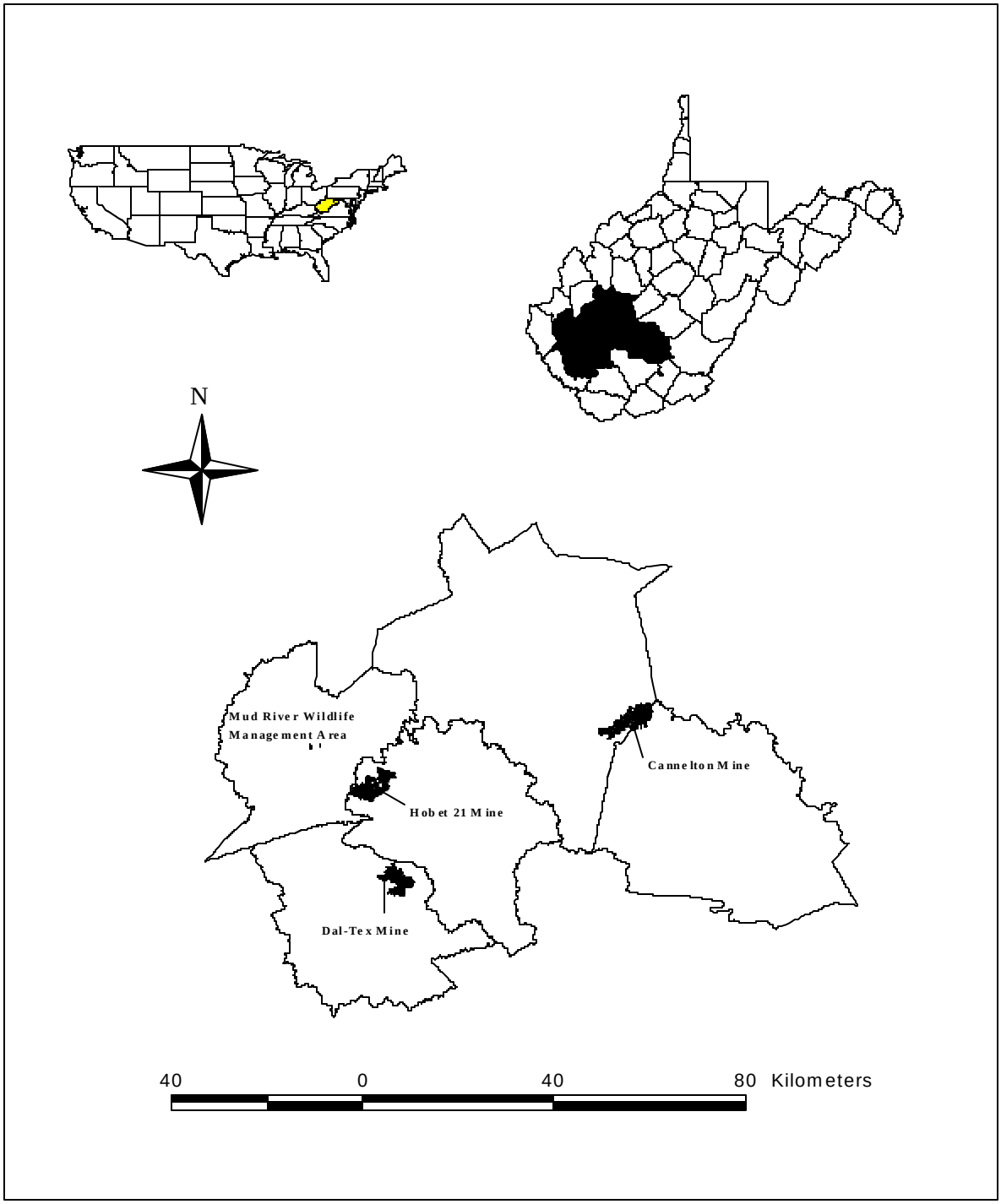
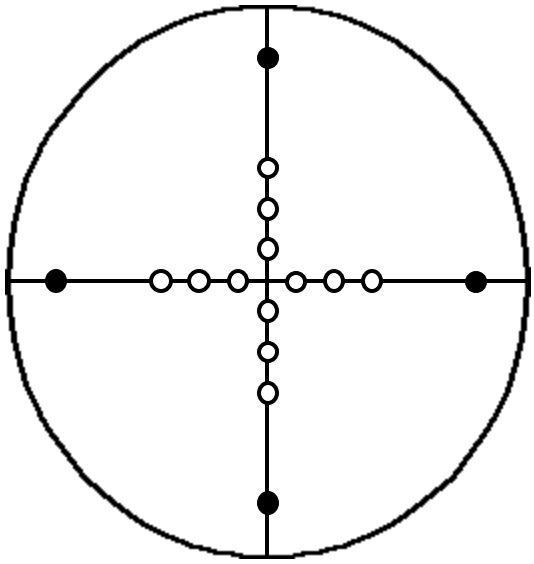
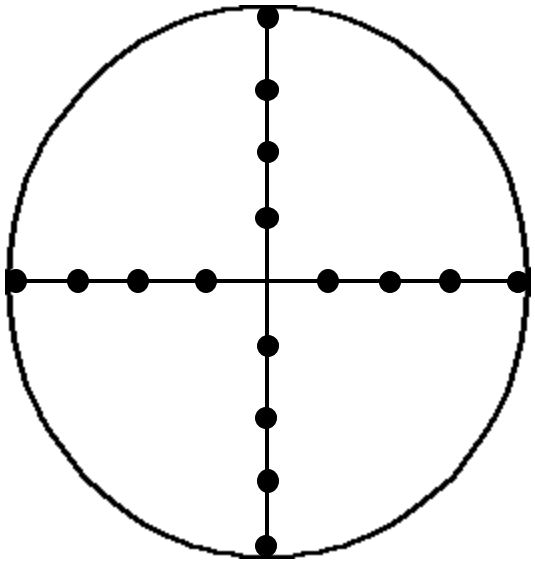


Figure 2. Diagram of the 11.3 m radius vegetation sampling plots used for Robel pole measurements, grass height measurements, litter depth measurements, vegetative cover estimates, and species occurrence observations on nest and habitat plots.



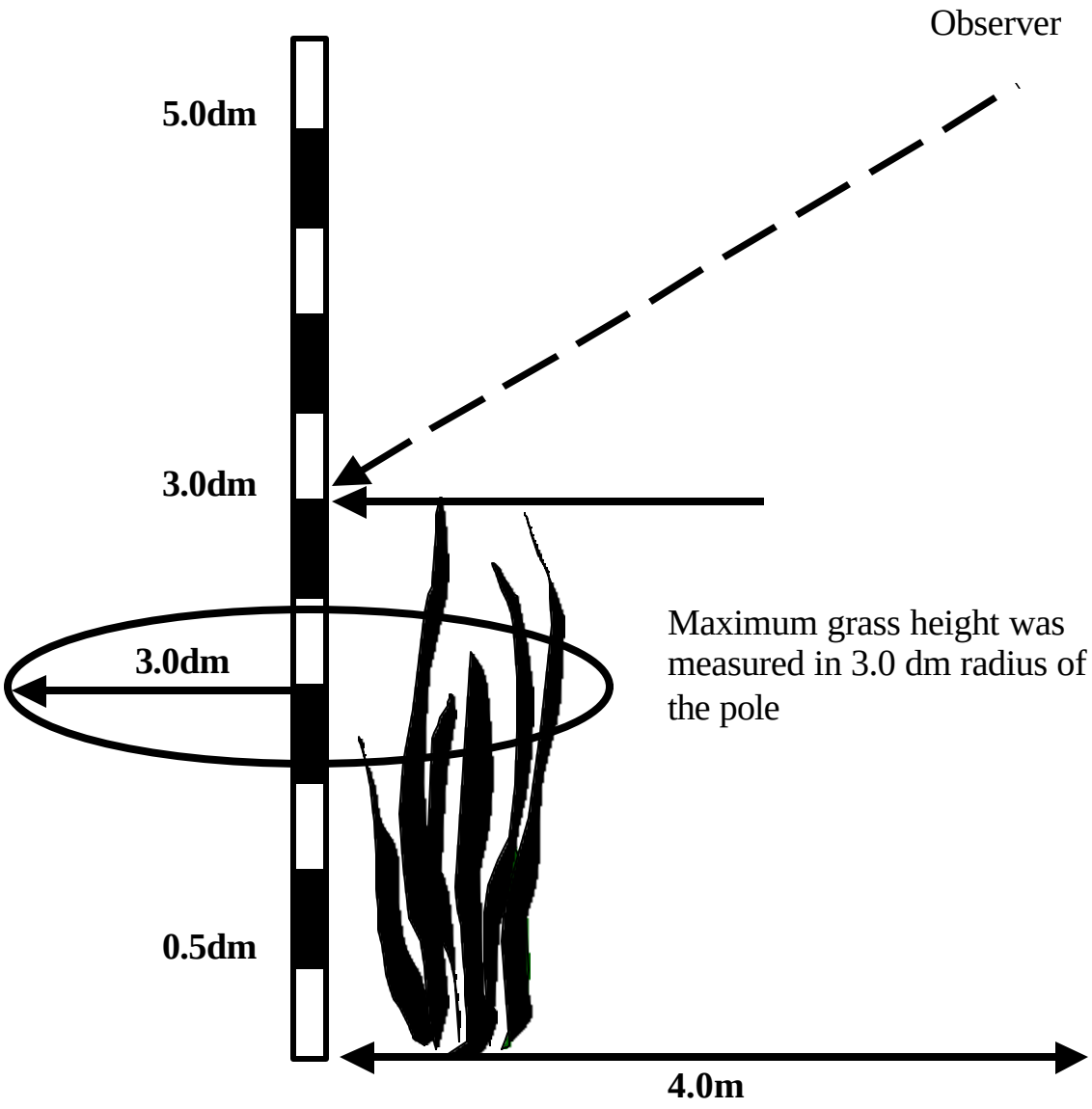


○ Robel Pole measurements

○ ● Grass height and litter depth

● Species occurrence observations and ground cover estimates

Figure 3. Diagram of Robel pole methods used to estimate an index of biomass. Cover was estimated 1.0 m above ground level facing toward the center of the plot. Observer stood 4.0 m away and recorded the last 0.5 dm interval not completely obscured by vegetation.



Chapter 3. Microsatellite DNA Analysis of Parentage, Kinship, and Population Assignment in Grasshopper Sparrow (*Ammodramus savannarum pratensis*) Populations Breeding in West Virginia

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ABSTRACT

I used polymorphic microsatellite DNA to examine genetic variation, mating system type, extra-pair paternity, parentage, and kinship within and among grasshopper sparrow populations inhabiting reclaimed mountaintop mining / valley fill complexes in southern West Virginia. Genetic differentiation within and among populations was quantified using two different fixation indices (Fst and Rst). I assessed parentage, kinship, and population assignment using likelihood-based approaches. The microsatellite markers examined in this study detected considerable genetic variation within and among grasshopper sparrow populations. Overall mean heterozygosity was near 67% with a mean number of 15 alleles per locus across all populations. Grasshopper Sparrow populations displayed low but significant differentiation among mine complexes while the genetic structure of breeding assemblages within mine complexes appears to be homogeneous. The results of the assignment test also suggest some differentiation at the population level and mine subpopulation level. Likelihood analyses of 161 adults and 51 juveniles identified maternity and paternity for 41 and 33 juveniles, respectively. I correctly identified parental pairs of 15 broods (36 nestlings) and the maternal parents of the remaining four nestlings. Extra-pair paternity was not observed in any of the broods examined. Data presented in this study may be used as a baseline to accurately monitor future population trends in this species. Populations examined on three reclaimed mines in this study do not appear to have low enough levels of genetic variation that would warrant management efforts directed at population genetic structure. Populations were panmictic suggesting that no specific areas on these complexes are critical for maintaining genetically diverse populations.

INTRODUCTION

Long-term population declines of migratory birds breeding in North America have become a major focus of studies addressing avian biodiversity, conservation, and management (Robbins et al. 1989, Askins et al. 1990, Vickery and Herkert 2001). Several grassland specialists breeding in North America have shown widespread and consistent population declines over the last century (Robbins et al. 1989, Askins 2000). While causal mechanisms responsible for declines are still largely unknown (James et al. 1992, Donovan and Flather 2002), agricultural intensification, loss or fragmentation of suitable grasslands, inadequate management of remaining habitats, disruption of natural disturbance regimes, and the degradation of wintering grounds have been implicated as potential causes (Kershner and Bollinger 1996, Herkert 1994, Johnson and Igl 2001, Vickery and Herkert 2001).

Polymorphic genetic markers have become a popular resource for researchers investigating ecological and evolutionary processes that influence population dynamics (Avise 1994). Microsatellite markers present several advantages in these types of studies compared to allozymes, restriction fragment length polymorphisms (RFLP's), minisatellites, and randomly amplified polymorphic DNA (Queller et al. 1993) and have been used for a variety of organisms including birds (Ellegren 1992, Taylor et al. 1994, Paetkau et al. 1995, Gibbs et al. 1997, Gibbs et al. 2000, Milot et al. 2000, Lee et al. 2001, Adcock and Mulder 2002, Castleberry et al. 2002 Coltman et al.2003).

The use of population structure data derived from genetic markers is based on the fact that polymorphic markers may be used to distinguish among individuals and populations (Smouse and Chevillon 1998). To gain a more complete understanding of dispersal, fine-scale population differentiation, kinship, social structure, species status, and phylogenetic relationships in birds, several studies have incorporated the use of poly-allelic microsatellite DNA loci (Ellegren 1992, Gyllenstein et al. 1989, Westneat 1990, Dawson et al. 1997, Alderson et al. 1999, Gibbs et al 2000, Lee et al. 2001, Tarof et al 2001, Bulgin et al. 2003). Several newly developed estimators of pairwise relatedness coefficients using co-dominant genetic markers (Queller and Goodnight 1989, Goodnight and Queller 1999, Lynch and Ritland, Wang 2002, Duchesne et al. 2002, Hardy 2003) have improved the efficiency and precision of studies focused on pedigree relationships.

The grasshopper sparrow (*Ammodramus savannarum*), a grassland specialist, is a broadly distributed passerine consisting of 12 recognized subspecies (Vickery 1996). Four of these subspecies, the eastern (*A. s. pratensis*), Florida (*A. s. floridanus*), western (*A. s. perpallidus*), and Arizona (*A. s. ammolegus*) breed in the continental United States (Vickery 1996, Rising 1996, Beadle and Rising 2002). Grasshopper sparrows inhabiting the eastern United States have experienced yearly population declines of 6% between 1966 and 2000 (Saur et al. 2002) and have been listed as a priority species throughout the Northeast (Donovan et al. 2002).

The nonmigratory Florida subspecies has experienced substantial declines due to habitat destruction and fragmentation (Delany et al. 2000, Shriver and Vickery 1999) resulting in this subspecies receiving official endangered species status in 1986 (Federal Register 1986). Delany et al. (2000) examined the levels of genetic variation within and among the six remaining *floridanus* populations to provide a basis for future monitoring and conservation efforts. Bulgin et al. (2003) used both mtDNA and microsatellite DNA to examine genetic variation and phylogenetic relationships among three of the North American grasshopper sparrow subspecies to assess the genetic distinctiveness of the *floridanus* race relative to the less threatened groups. To date, no genetic analysis has been conducted to investigate genetic differentiation and relatedness within and among declining grasshopper sparrow populations inhabiting grassland areas in the eastern United States.

Mountaintop mining / valley fill (MTMVF) operations in southern West Virginia convert large areas of mature mixed mesophytic forest to early successional habitats creating potential habitat for grassland songbird populations. Grasshopper sparrows are the most abundant species on these novel grassland sites (Wood et al. 2001), however, little is known about these populations on a molecular level. These large grassland islands present a unique opportunity to include both demographic and genetic data at various scales to gain insight into the breeding biology and population dynamics of grasshopper sparrows.

The specific aims of this study are to: (1) assess population differentiation and structure within and among populations; (2) determine if grasshopper sparrow assemblages on each mine complex are distinct breeding sub-populations or one large panmictic population; (3) examine mating system behavior to determine the incidence of extra-pair paternity (EPP) in this species; (4) examine parentage and kinship relationships within populations; and, (5) examine the utility of the genetic markers used in this study for population assignment.

MATERIALS AND METHODS

Sample collection

A total of 415 grasshopper sparrows were captured and processed from six areas on three Mountaintop Mining/Valley Fill (MTMVF) complexes and one non-mined reference area in southwestern West Virginia during the 2001 and 2002 breeding seasons (Table 1). Two geographically distinct grasshopper sparrow assemblages were identified on each mine complex to ensure a spatially representative sample of each mine population. Grasshopper sparrows were captured with mist nets using conspecific song playback from April to August 2001-2002. All captured individuals were banded with U. S. Fish and Wildlife Service leg bands and a unique combination of two colored plastic leg bands for future visual identification. Gender and age of captured individuals was determined using plumage differences, physical measurements, and breeding characteristics (cloacal protuberance and brood patch) (Pyle et al. 1997). When possible, offspring were captured and processed prior to fledging (5-6 days post hatch) and banded with a USFWS leg band and a single colored leg band.

In 2001, head and breast feather samples were collected from all individuals according to the methods of Taberlet and Bouvet (1991). In 2002, approximately 50 µl of blood was obtained from the tarsal vein of each individual and stored in 1 ml of a standard lysis buffer (Seutin et al. 1991) at –20 °C. Care was taken to ensure that bleeding had stopped prior to release. All captured individuals were released immediately following the sampling procedures.

DNA isolation

Several DNA isolation methods (Taberlet and Bouvet 1991, QIAGEN 1998) for animal tissue were compared for optimal DNA yield and purity from feather samples (Ammer and Gatesman, unpubl. data). Feather samples did not produce DNA sufficient for amplification and subsequent microsatellite analyses and were not considered further. DNA from 212 blood samples obtained in 2002 was extracted using a DNeasy Tissue Kit (Qiagen) and stored at 4°C. Spectrophotometric analysis was performed on each sample at 260 nm and 280 nm to quantify yield and ensure proper DNA concentrations necessary for amplification by polymerase chain reaction (PCR).

Microsatellite analysis

PCR methodology

Six microsatellite loci Cuμ02 (Gibbs et al. 1999), Asμ09, Asμ15, Asμ18 (Bulgin et al. 2003), Maμ23 (Alderson et al. 1999), and Dpμ16 (Dawson et al. 1997) (Table 2) were found to be polymorphic in grasshopper sparrows (Bulgin et al 2003) and were assessed to determine if they possess adequate variability to determine parentage. Microsatellite loci were amplified by PCR using modified methods of Bulgin et al. (2003). Briefly, reactions for each locus were carried out in 10 μl volumes using 7 pmol of each primer (forward primers labeled with FAM, HEX, or NED; Applied Biosystems), 10 μM of each dNTP, 2.0 mM $MgCl_2$, 1X PCR reaction buffer, 50 ng of DNA template, and 0.25 units of Taq polymerase (New England BioLabs). The following PCR profile consisted of an initial denaturing step of two minutes at 94°C followed by 45 cycles of 94°C denaturing step for 30 s, published annealing temperature for each primer set for 30 s, and a 72°C extension step for 30 s. Samples were held for extension at 72°C for 10 minutes and stored at 4°C. Thermal cycling was performed in a MJ DNA Engine (PTC 200, MJ Research) configured with a heated lid.

Capillary electrophoresis and sample scoring

For all analyses, 1 μl of each PCR product was diluted (1:100) with deionized water. Then, 1 μl of each PCR dilution was added to a 12 μl mixture (1:100) of deionized formamide and internal size standard GENESCAN-GS400HD (Applied Biosystems). The resulting 13 μl mixture was heat denatured at 95 °C for 3 min and placed on ice for at least 5 min. The PCR product/formamide/size standard mixture was subjected to capillary electrophoresis on an Applied Biosystems PRISM 310 Genetic Analyzer. I used GENESCAN and GENOTYPER (version 2.0) software packages (Applied Biosystems) to score the fluorescently labeled DNA fragments and generate allelic and genotypic designations for each grasshopper sparrow.

Statistical analyses

Genetic polymorphism

Genetic diversity was measured for each mine population and at each locus as the number of alleles per locus (A) and expected and observed heterozygosity (H_e and H_o, respectively).

Estimates of allelic and genotypic frequency distribution were generated for each population and sub-population at each locus. Genotypic linkage disequilibrium was calculated with the GENEPOP software package, v 3.1c (Raymond and Rousset 1995) to examine physical linkage of all loci considered. Hardy-Weinberg equilibrium (HWE) exact tests were calculated with GENEPOP using the Markov chain randomization test (Guo and Thompson 1992) to estimate 2-tailed P-values for each locus in each sample. I also tested for significant heterozygote deficiency per locus and population using the heterozygote deficiency subroutine in GENEPOP (Raymond and Rousset 1995). Critical values were adjusted to account for multiple tests of the same hypothesis using the Bonferroni adjustments (Rice 1989).

Population structure

Because mutation processes in microsatellites are unclear, genetic differentiation within and among populations was quantified using two different fixation indices (F_{st} and R_{st}) (Balloux and Lugon-Moulin 2002). We estimated F_{st} with FSTAT (Goudet 1995) and R_{st} with RSTCALC (Goodman 1997), using permutation procedures in both packages to test whether calculated values differed significantly from zero. F_{st} estimates were calculated assuming an infinite alleles model (IAM; Kimura and Crow 1964) to generate theta (θ; Weir and Cockerham 1984). R_{st} was estimated assuming a stepwise mutation model (SMM; Kimura and Otha 1978) to generate rho (ρ; Slatkin 1995). Critical values were adjusted to account for multiple tests of the same hypothesis using the Bonferroni adjustments (Rice 1989).

Parentage and kinship

A total of 31 candidate females, 130 candidate males, and 51 offspring were assessed using the software package PAPA (Duchesne et al. 2002) and the CERVUS software package (Marshall et al. 1998) to examine parental relationships. The PAPA package allocates parentage based on the breeding likelihood of a parental pair producing the genotype observed in a given offspring. Parentage analysis with this allocation method allows for some error due to genotype misreading and mutation. The CERVUS package uses a likelihood-based approach to infer paternity.

Relatedness at the subpopulation and population level was examined with the software program Kinship (Goodnight and Queller 1999) to determine the amount of relatedness between pairs of individuals within each assemblage. This program uses specified r values, population

allele frequencies, and the genotypes of the two individuals being compared to calculate the likelihood that this genotype combination was produced by the specified relationship (Goodnight and Queller 1999).

Assignment test

Assignment tests were conducted to determine the likelihood of each individual's genotype being found in the group from which it was collected using the program GENECLASS (Cornuet et al. 1999). Assignment calculations used multilocus likelihood functions (Paetkau et al. 1995) to classify individuals based on the highest likelihood probability or the lowest likelihood distance using the Bayesian method.

RESULTS

Microsatellite variation

Multilocus genotypes at six microsatellite loci were generated for 212 grasshopper sparrows that colonized seven grassland sites in southern West Virginia. The Caμ02 locus was excluded from all analyses due to inconsistencies in amplification and allele scoring. Genetic characteristics of the five microsatellite loci surveyed in this study are summarized in Table 3. A total of 75 alleles were observed across five loci and ranged from nine at Maμ23 and Dpμ16 to 22 at Asμ15. The mean number of alleles per locus across all populations was 15. Allele size range (bp) differences between the smallest and the largest allele per locus ranged from 16 bp (Mau23) to 42 bp (Asu15, Asu18). A total of 11 unique alleles were observed among populations, four in the Dal-Tex population, six in the Hobet 21 population, and one in the Cannelton population. No unique alleles were observed from the MRWMA population. Allele frequencies of all loci in all populations are described in Table 4. Overall mean heterozygosity was 0.67 and ranged from 0.66 on the Hobet 21 mine to 0.70 on the Dal-Tex mine.

Two-tailed tests for departure from Hardy-Weinberg equilibrium ($P<0.05$) indicated significant deviation at one locus in Dal-Tex (Asu09), Cannelton (Dpμ16) and MRWMA (Dpμ16). Additional multilocus tests for heterozygote deficit detected two subpopulations (CLF, DSF) with significant heterozygote deficiencies ($P<0.007$). After I applied the Bonferroni adjustment for multiple comparisons, no significant differences were observed. The absence of significant heterozygote deficiency in all loci within a population suggests little inbreeding in

these mine populations. The lack of significant heterozygote deficiencies at the same locus across populations implies that null alleles were at low frequencies if present. Null alleles are due to mutations in the primer sites of individuals and result in scoring a true heterozygote as a homozygote because only one of the two alleles present will be amplified. The presence of nonamplifying or null alleles may result in erroneous parental allocation, kinship designation, and population assignment. Genotypic disequilibrium exact tests revealed no significant values ($P<0.05$) indicating that physical linkage of loci is unlikely.

Population Structure

The overall è-value was significant (è=0.010; $P<0.001$) and significant values of F_{st} were observed among mine populations (Table 5), however, no differences were detected between any of the mine populations and the MRWMA. Results obtained from pairwise comparisons using ρ (R_{ST}) are similar to those generated by è (ρ=0.007; $P<0.001$) (Table 5). Pairwise comparisons of è (F_{ST}) and ρ (R_{ST}) values indicated no significant differentiation within populations on each mine suggesting that individual mine assemblages are single panmictic groups (è=0.008; ρ=0.006).

Parentage assignment and kinship

Likelihood analyses of 161 adults and 51 juveniles identified maternity and paternity for 41 and 33 juveniles, respectively. We were able to correctly identify parental pairs of 15 broods (36 offspring) and the two maternal parents of the remaining four nestlings. The 15 remaining juveniles that could not be assigned both parents were assessed using pairwise relatedness comparisons ($P<0.05$) to determine potential sibling groups. Eleven unassigned juveniles were placed in 5 distinct sibling groups, while four individuals were identified as unrelated to any other juveniles.

Parentage in 17 grasshopper sparrow broods comprised of two or more offspring was examined to determine the incidence of extra-pair paternity in these populations. I observed no incidence of extra-pair paternity in any of the broods surveyed.

Altruistic behavior at six nests by eleven non-parental individuals was examined to determine the relationships between the helpers and the territorial breeding pair. Eight nest helpers were

identified as either maternal and/or paternal offspring and three adult females were identified as unrelated ($P<0.05$).

Population assignment

Assignment of individuals to mine populations and subpopulations (plots) suggests some subdivision at both levels. Mean correct classification rate at the population level was 77%, ranging from 14% at MRWMA to 96% on the Cannelton mine. Mean correct assignment at the subpopulation level was 66% and ranged from 56% on the Hobet 21 Sugartree Branch site to 92% on the Cannelton Cabin Creek site (Table 6).

DISCUSSION

Grasshopper Sparrow populations surveyed in this study displayed low but significant differentiation among mine complexes while the genetic structure of breeding assemblages within mine complexes appear to be homogeneous. Weak differentiation among populations is relatively common in avian species, with high dispersal rates being the most likely explanation (Dawson et al. 1997, Lee et al.2001). Bulgin et al. (2003), using the same loci used in this study, observed a lack of genetic differentiation among eastern grasshopper sparrow populations sampled in Georgia, Ohio, and Massachusetts suggesting that some gene flow occurs among these migratory populations.

The results of the assignment test show evidence of some genotypic differentiation at the population level (mine and WMA) and mine subpopulation level. These analyses are based on genotype data (Paetkau et al. 1995) and offer an added perspective to the more commonly used methods that rely on allele frequencies (Slatkin 1995). Correct assignment at the population level exceeded 77% using the five microsatellite screened in this study. Correct assignment of individuals at the subpopulation level was lower and is most likely due to the fact that grasshopper sparrow assemblages on mine complexes are panmictic.

The five polymorphic microsatellite loci screened in this study are robust and appear to be effective in allocating parentage when neither parent is known. I was successful at assigning at least one parent to 80% of the offspring surveyed using maximum likelihood methods. Most correct parental allocations occurred among offspring and candidate parents that were sampled

from the same mine population suggesting little or no movement among groups during the breeding season. However, all juveniles inhabiting the MRWMA were allocated to females that were collected on the Hobet 21 mine complex. Paternal parents of the offspring were identified as the two adult males that were collected on the MRWMA site. These individuals were collected late in the breeding season (7/28/02 and 8/9/02) and may have moved to this area from the Hobet 21 complex prior to migration. No nests or other indications of breeding were detected on the MRWMA (Chapter 2). Accurate parentage assessment will ultimately contribute to the determination of the genetic payoff associated with observed behavioral strategies and lifetime reproductive success.

I screened five polymorphic microsatellite loci for this study. The addition of more microsatellite loci may improve the overall precision of these analyses by reducing the impact of genotyping errors on parental allocation (Bernatchez and Duchesne 2000, Duchesne et al. 2002). Incomplete sampling of candidate parents and offspring also may confound parentage resolution and should be considered when choosing parentage models (Marshall et al. 1998, Neff et al. 2000). Alderson et al. (1999) suggested that linkage between loci, the presence of null alleles, and mutation events may reduce the sensitivity of microsatellite markers in parentage analyses.

The occurrence of extra-pair paternity (EPP) in avian species was thought to be rare in avian mating systems (Lack 1968). The advent of molecular tools has increased parentage resolution to reveal that true monogamy occurs in only about 14% of passerine species that have been surveyed (Griffith et al. 2002), however, the percentage of EPP has been found to vary greatly both within and among species (Møller and Ninni 1998). Several recent efforts have examined environmental factors that may contribute to observed EPP variation (Petrie and Kempenaers 1998) and also the evolutionary costs and benefits of this behavior (Krokene et al.1998). The lack of extra-pair paternity within the grasshopper sparrow broods that I sampled implies a socially and genetically monogamous mating system in this species. In all cases, paternity was assigned to the male that was commonly observed defending the territory in which the nest was located. Care should be taken when interpreting this result mainly due to the spatial and temporal scale of this study and small number of complete broods for which both parents were correctly assigned.

Altruistic nest helping behavior has been previously described in this species (Kaspari and O'Leary 1988); however, the authors only speculate on the relatedness among the

participants. I found that 8 of the 11 individuals observed delivering food items to the brooding female were non-reproductive offspring (3 male, 5 female) of at least one of the breeding pair. The remaining three nest helpers were reproductive females from neighboring territories and were unrelated to either of the breeders. Cooperation among unrelated individuals in migratory species raises questions pertaining to the cost/ benefit ratio associated with these adaptive behaviors (Ligon 1983) and also the degree of reciprocity among cooperators.

Conservation and management implications

The levels of genetic polymorphism in eastern grasshopper sparrow populations examined by Bulgin et al. (2003) are comparable to observed levels in breeding populations in West Virginia. Populations examined on three reclaimed mines in this study do not appear to have low enough levels of genetic variation that would warrant management efforts directed at population genetic structure. Populations were panmictic suggesting that no specific areas on these complexes are critical for maintaining genetically diverse populations. Data presented in this study and by Bulgin et al. (2003) may be used as a baseline to accurately monitor future population dynamics in this species.

I captured many adult male birds in breeding condition that were not confirmed territory holders and were not assigned paternity to the juveniles examined in this study. I expected to capture more reproductive males than females and juveniles because I used conspecific song to attract birds to the mist nets. I also observed many marked males moving among available grassland habitats on the mine complexes suggesting that floaters may comprise a segment of these populations. An alternative explanation is that I did not locate all of the nests and offspring related to these presumably unmated individuals.

Future research efforts should examine rangewide variation within *Ammodramus savannarum* subspecies to assess the amount of gene flow present among breeding assemblages on multiple scales. Dispersal mechanisms, the genetic composition of non-breeding winter assemblages, and site fidelity are poorly quantified in this species (Vickery 1996) and may offer insight into the magnitude of observed differentiation among sampled populations.

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Table 1. Site locations, area of the complex, plot code, plot size, site name, and sample size (N) for seven grasshopper sparrow assemblages sampled in southern West Virginia in 2001-2002.

Site	Area (ha)	Plot Code	Plot Size (ha)	Plot Name	N
Cannelton Mine	2180				
		CLF	36.1	Lynch Fork	13
		CCC	28.0	Cabin Creek	25
Dal-Tex Mine	1925				
		DRH	45.1	Rockhouse Creek	18
		DSF	40.0	Spruce Fork	31
Hobet 21 Mine	2431				
		HAF	51.3	Adkins Fork	48
		HST	50.9	Sugartree Branch	70
Mud River Wildlife Management Area	104	WMA	23.0	Mud River	7

Table 2. Published sequences, reported repeat motif, number of published alleles (*A*), annealing temperature (AT) of forward and reverse primers for microsatellite loci used in this study.

Locus	Size (bp)	*A*	Repeat Motif	AT	Primer Sequence (5' 3')	Study
*Cu*µ02F	146	10	$(TG)_{15}$	60	F: CCTTGGATTGCTTCCAAATG	Gibbs et al. 1999
*Cu*µ02R					R: CCAATTTCCTGCAGACTCTTTC	
*Dp*µ16F	162	18	$(AC)_{12}(GC)_4ACGCAC(GC)_2$	50	F: ACAGCAAGGTCAGAATTAAA	Dawson et al. 1997
*Dp*µ16R					R: AACTGTTGTGTCTGAGCCT	
*As*µ09F	153	15	$(CA)_{24}$	55	F: CTTTGATTACAGAAATATGTCTTCT	Bulgin et al. 2003
*As*µ09R					R: GAAAGAGGCATGCTCGTAT	
*As*µ15F	135	18	$(TG)_9$	53	F: AATAGATTCAGGTGCTTTTTC	Bulgin et al. 2003
*As*µ15R					R: TAGCACATGTTGGTTTTTG	
*As*µ18F	132	21	$(AC)_7TC(AC)_9$	53	F: ACACAGAGAGACACAAATTCAT	Bulgin et al. 2003
*As*µ18R					R: AAATGCTACTGAGGTAAAGTCC	
*Ma*µ23F	157	9	$(TG)_3(T)_3(TG)_{15}$	61	F: TGCCAGTATTCTCTTGTGCTT	Alderson et al. 1999
*Ma*µ23R					R: CTGTGGGATGTAGGAATTGTG	

Table 3. Summary of grasshopper sparrow microsatellite polymorphism for populations and subpopulations sampled in West Virginia in 2002. Variables are number of individuals captured (N), expected and observed heterozygosity (He and Ho, respectively), and total number of alleles (*A*) per locus.

	Cannelton Mine			Dal-Tex Mine			Hobet 21 Mine			MRWMA	
Locus	CLF	CCC	Combined	DSF	DRH	Combined	HA1	HST	Combined		Total
Asμ09											
N	13	25	38	31	18	49	48	70	118	7	212
H_e	0.86	0.86	0.82	0.82	0.85	0.85	0.85	0.80	0.82	0.67	0.83
H_o	0.69	0.80	0.84	0.61	0.61	0.74	0.71	0.74	0.64	0.71	0.70
A	11	9	12	12	8	13	14	13	14	5	15
Asμ15											
N	13	25	38	31	18	49	48	70	118	7	212
H_e	0.93	0.81	0.91	0.93	0.92	0.90	0.92	0.92	0.91	0.78	0.91
H_o	0.92	0.84	0.92	0.87	0.83	0.90	0.92	0.87	0.86	0.86	0.88
A	12	11	15	16	14	17	18	19	21	7	22
Asμ18											
N	13	25	38	31	18	49	48	70	118	7	212
H_e	0.89	0.85	0.87	0.86	0.82	0.88	0.90	0.86	0.87	0.67	0.87
H_o	0.77	0.88	0.76	0.77	0.88	0.90	0.79	0.84	0.81	0.71	0.82
A	13	14	15	17	12	18	17	16	18	8	20
Dpμ16											
N	13	25	38	31	18	49	47	70	117	7	211
H_e	0.77	0.69	0.59	0.47	0.47	0.62	0.69	0.60	0.64	0.53	0.62
H_o	0.54	0.60	0.53	0.48	0.50	0.51	0.69	0.51	0.59	0.57	0.56
A	6	7	9	5	6	6	7	6	8	3	9
Maμ23											
N	13	25	38	31	18	49	48	70	118	7	212
H_e	0.22	0.29	0.40	0.44	0.31	0.41	0.38	0.47	0.37	0.52	0.35
H_o	0.23	0.32	0.42	0.48	0.32	0.45	0.35	0.51	0.38	0.57	0.37
A	4	5	5	7	5	7	4	6	6	2	9

Table 4. Allele frequencies listed by fragment length of five microsatellite loci examined in grasshopper sparrow populations breeding in southern West Virginia, 2002.

	Sampled Population				
Locus/allele	Cannelton	Dal-Tex	Hobet	WMA	Overall
Asu09					
119	0.000	0.010	0.000	0.000	0.002
121	0.066	0.020	0.017	0.000	0.026
123	0.066	0.051	0.089	0.143	0.078
125	0.303	0.306	0.369	0.500	0.347
127	0.105	0.184	0.110	0.000	0.123
129	0.105	0.082	0.089	0.071	0.090
131	0.079	0.122	0.097	0.000	0.097
133	0.118	0.092	0.034	0.214	0.068
135	0.013	0.010	0.008	0.000	0.009
137	0.066	0.010	0.051	0.000	0.042
139	0.000	0.000	0.047	0.000	0.026
141	0.053	0.051	0.038	0.071	0.045
143	0.013	0.020	0.038	0.000	0.028
145	0.000	0.000	0.008	0.000	0.005
147	0.013	0.041	0.004	0.000	0.014
N	38	49	118	7	212
Asu15					
107	0.000	0.000	0.008	0.000	0.005
109	0.000	0.010	0.004	0.000	0.005
111	0.000	0.061	0.017	0.000	0.024
113	0.000	0.000	0.008	0.000	0.005
115	0.000	0.000	0.008	0.000	0.005
117	0.013	0.000	0.013	0.000	0.009
119	0.039	0.020	0.072	0.000	0.052
121	0.013	0.000	0.034	0.000	0.021
123	0.184	0.112	0.076	0.286	0.111
125	0.250	0.133	0.106	0.000	0.134
127	0.039	0.061	0.034	0.143	0.045
129	0.026	0.092	0.055	0.000	0.057
131	0.211	0.143	0.153	0.000	0.156
133	0.053	0.061	0.042	0.071	0.050
135	0.039	0.041	0.127	0.214	0.094
137	0.000	0.041	0.025	0.071	0.026
139	0.013	0.051	0.097	0.071	0.071
141	0.053	0.020	0.055	0.143	0.050

Table 4. Cont.

143	0.026	0.051	0.034	0.000	0.035
145	0.026	0.051	0.021	0.000	0.028
147	0.000	0.031	0.008	0.000	0.012
149	0.013	0.020	0.000	0.000	0.007
N	38	49	118	7	212
Dpu16					
145	0.026	0.000	0.004	0.000	0.007
149	0.026	0.010	0.034	0.000	0.026
151	0.211	0.071	0.145	0.143	0.140
153	0.474	0.714	0.556	0.50	0.576
155	0.092	0.143	0.145	0.357	0.142
157	0.066	0.031	0.085	0.000	0.066
159	0.053	0.031	0.017	0.000	0.026
161	0.039	0.000	0.013	0.000	0.014
163	0.013	0.000	0.000	0.000	0.002
N	38	49	117	7	211
Asu18					
105	0.013	0.010	0.008	0.000	0.009
107	0.000	0.020	0.030	0.000	0.021
109	0.039	0.071	0.025	0.071	0.040
111	0.329	0.286	0.288	0.143	0.290
113	0.079	0.041	0.047	0.143	0.054
115	0.079	0.061	0.089	0.143	0.083
117	0.039	0.020	0.068	0.071	0.052
119	0.079	0.071	0.038	0.214	0.059
121	0.079	0.214	0.106	0.000	0.123
123	0.026	0.010	0.064	0.000	0.042
125	0.118	0.041	0.064	0.000	0.066
127	0.000	0.010	0.030	0.000	0.019
129	0.013	0.061	0.055	0.000	0.047
131	0.026	0.010	0.021	0.000	0.019
133	0.026	0.031	0.021	0.071	0.026
137	0.026	0.010	0.030	0.000	0.024
139	0.026	0.000	0.004	0.000	0.007
141	0.000	0.000	0.013	0.143	0.012
145	0.000	0.010	0.000	0.000	0.002
147	0.000	0.020	0.000	0.000	0.005

Table 4. Cont.

N	38	49	118	7	212
Mau23					
144	0.000	0.000	0.004	0.000	0.002
146	0.000	0.010	0.000	0.000	0.002
148	0.855	0.776	0.737	0.857	0.771
150	0.026	0.051	0.072	0.000	0.057
152	0.053	0.061	0.119	0.000	0.090
154	0.026	0.061	0.059	0.143	0.057
156	0.000	0.031	0.008	0.000	0.012
158	0.039	0.000	0.000	0.000	0.007
160	0.000	0.010	0.000	0.000	0.002
N	38	49	118	7	212

Table 5. Matrix of genetic variation among mine populations of grasshopper sparrows. (F_{ST} values above the diagonal and R_{ST} values below the diagonal). All F_{ST} (è) and R_{ST} (ρ) values in bold, are significantly different from zero after Bonferroni correction (P<0.008).

	Cannelton	Dal-Tex	Hobet 21	MRWMA
Cannelton	—	**0.0013**	**0.008**	0.029
Dal-Tex	**-0.004**	—	**0.007**	0.026
Hobet 21	**-0.003**	**-0.007**	—	0.015
MRWMA	-0.015	-0.032	-0.028	—

Table 6. Results of maximum-likelihood assignment tests from seven grasshopper subpopulations. Within each column, boxes represent the total number of correct assignments for that population. The total number of classifications or sample size is represented by *n*, CCOS and CCOP is the number of correct classifications observed at the subpopulation and population levels respectively, and %CCS and %CCP is the percentage of correct classifications at each level. Location abbreviations refer to study populations described in Table 1.

	CLF	CCC	DSF	DRH	HST	HAF	WMA
CLF	11	1	0	0	8	3	0
CCC	1	23	1	0	4	5	1
DSF	1	1	22	1	6	3	0
DRH	0	0	2	16	2	1	1
HST	0	0	4	1	39	8	4
HAF	0	0	2	0	11	28	0
WMA	0	0	0	0	0	0	1
n	13	25	31	18	70	48	7
CCOS	11	23	22	16	39	28	0
%CCS	85	92	71	89	56	58	0
CCOP	12	24	24	17	50	36	1
%CCP	92	96	77	94	70	75	14

Chapter 4. Gender Determination of Grasshopper Sparrows By Application of Morphological and Molecular Techniques

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ABSTRACT

Accurate gender determination in monomorphic species is often difficult especially if individuals do not display behavioral and breeding characteristics. I describe the efficiency and accuracy of gender determination in the monomorphic grasshopper sparrow with morphological characters and recently developed DNA sexing techniques. Gender was determined in all 211 individuals examined with the 2550F/2718R primer set and 3% agarose gel electrophoresis. Gender data collected in the field using breeding characteristics matched the DNA analyses with 100 % accuracy for adult males and females. Gender was determined for all captured juvenile individuals that did not display sex-linked traits or behaviors in the field. The male to female sex ratio of the offspring examined in this study is slightly skewed (64:36 male to female) but was not statistically different from a 50:50 sex ratio. Gender determination of adult individuals not displaying secondary sexual characteristics and behaviors is still problematic and cannot be resolved accurately with traditional morphological and behavioral cues. The molecular techniques used in this study offer a high level of accuracy and may be used to investigate dispersal mechanisms and winter assemblage composition in monomorphic species.

INTRODUCTION

Accurate gender determination of adult and juvenile birds is often difficult in demographic studies examining migratory species with few or no dimorphic characters. In species with no size or plumage dimorphism, gender can be reliably determined only during the breeding season when behavioral and external sexual characteristics can be directly observed (Underwood et al. 2002). Determining the gender of juveniles presents additional difficulty because these individuals display no sex-linked traits or behaviors. The ability to accurately assign gender is especially useful when examining sex specific factors such as juvenile sex ratios and gender specific habitat selection (Underwood et al. 2002).

Until recently, the most reliable methods used in gender determination studies were invasive surgical procedures that required anesthesia and were not a feasible option for most field studies. The advent of polymerase chain reaction (PCR) techniques targeting the chromo-helicase DNA (CHD) binding gene (Griffiths et al. 1998, Fridolfsson and Ellegren 2000) provided a noninvasive alternative that is relatively simple and inexpensive to perform. The CHD gene occurs in two genetically distinct forms, the CHD W-linked form (*CHD-W*) and the CHD Z-linked form (*CHD-Z*). Because the *CHD-W* and *CHD-Z* genes occur on different sex chromosomes, gender can be determined by examining the composition of these genes (Boutette et al. 2002). In birds, the male is homogametic having two Z chromosomes, while the heterogametic female has one Z chromosome and one W chromosome (Fridolfsson and Ellegren 1999). Recently developed techniques rely on PCR primers that anneal to the conserved exonic regions and also amplify across an intron in both genes (Griffiths et al. 1998, Fridolfsson and Ellegren 1999, Dawson et al. 2001). These techniques exploit the fact that the less conserved noncoding introns in the *CHD-Z* and *CHD-W* genes vary in length and therefore can be differentiated by gel electrophoresis (Griffiths et al. 1998).

The Grasshopper Sparrow (*Ammodramus savannarum*) is a widely distributed North American grassland species that has shown long-term population declines over much of its range (Robbins et al. 1989). Individuals of the eastern subspecies (*A. s. pratensis*) display no plumage or size dimorphism making this group extremely difficult to sex by observation alone. Adult females in the breeding territory are secretive and commonly spend much time on the ground and walk to and from the nest area so not to draw attention to brooding and feeding behaviors

(Vickery 1996). Territorial male behaviors are much more obvious because they commonly perch and sing from perches at the periphery of the territory (Vickery 1996). Previous studies have indicated that some morphological and behavioral characteristics may be useful in gender determination in this species, however, quantitative evidence supporting the accuracy and applicability of these characters is lacking (Delany et al. 1994).

In this study, I compared the efficiency of the P2/P8 (Griffiths et al. 1998) and 2550F/2718R (Fridolfsson and Ellegren 1999) primer sets for accurate gender determination of adult and juvenile Grasshopper Sparrows. Data gained through DNA sexing was used to examine juvenile sex ratio relationships within broods. In addition to the molecular methods, I examined several morphological characters to determine their utility in accurate gender assessment in the field.

METHODS

Sample collection-A total of 413 grasshopper sparrows were captured and processed from three Mountaintop Mining/Valley Fill (MTMVF) complexes (Cannelton, Dal-Tex, Hobet 21) and one non-mined reference area (Mud River Wildlife management Area) in southwestern West Virginia (Figure 1) during the 2001 and 2002 breeding seasons. Two distinct grasshopper sparrow subpopulations were identified on each mine complex to ensure a temporally and spatially representative sample.

Grasshopper sparrows were captured with mist nets using conspecific song playback from April to August 2001 and 2002. All captured individuals were banded with U. S. Fish and Wildlife Service leg bands and a unique combination of two colored plastic leg bands for future visual identification. Mass, wing chord, bill width, bill length, and tarsus length were measured using the methods described in Pyle et al. (1997) for the 211 individuals captured in 2002. In 2001, only mass and wing chord were measured. Gender and age of captured individuals was determined in the field using behavioral observations, plumage differences (age), physical measurements, and breeding characteristics (cloacal protuberance and brood patch) (Pyle et al. 1997). When possible, offspring were captured and processed prior to fledging (5-6 days post hatch) and banded with a USFWS leg band and a single colored leg band.

Approximately 50 µl of blood was collected from 211 adult and juvenile Grasshopper Sparrows during the 2002 breeding season by femoral veinipuncture. Blood samples were stored in 1 ml of a standard blood lysis buffer (Seutin et al. 1990) at –20 °C. Care was taken to ensure that bleeding had stopped prior to release. All captured individuals were released immediately following sampling procedures.

DNA extraction and PCR amplification-Genomic DNA was extracted using a DNeasy Tissue Kit (Qiagen) and stored at 4°C. Spectrophotometer analysis was performed on each sample at 260 nm and 280 nm to quantify yield and ensure proper DNA concentrations necessary for amplification by PCR.

Reactions for P2/P8 primer set were amplified by PCR using modified methods of Griffiths et al. (1998) in 10 µl volumes using 100 ng of each primer, 200 µM of each dNTP, 2.0 mM $MgCl_2$, 1X PCR reaction buffer, 50 ng of genomic DNA template, and 0.25 units of *Taq* polymerase (New England BioLabs). The following PCR profile consisted of an initial denaturing step of two minutes at 94°C followed by 40 cycles of 94°C denaturing step for 45 s, 48°C for 45 s, and a 72°C extension step for 45 s. Samples were held for extension at 72°C for 10 minutes and stored at 4°C.

Reactions using the 2550F/2718R primer set were amplified by PCR using modified methods of Fridolfsson and Ellegren (1999). Briefly, reactions were carried out in 10 µl volumes using 1 µM of each primer, 200 µM of each dNTP, 2.0 mM $MgCl_2$, 1X PCR reaction buffer, 50 ng of genomic DNA template, and 0.25 units of *Taq* polymerase (New England BioLabs). The following PCR profile consisted of an initial denaturing step of two minutes at 94°C followed by a 94°C denaturing step for 45 s, 60°C for 45 s, and a 72°C extension step for 45 s. Annealing temperature was reduced 1°C per cycle until an annealing temperature of 51°C was reached. Samples were then cycled at 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s for 30 cycles. Samples were held for extension at 72°C for 10 minutes and stored at 4°C.

All thermal cycling was performed in a MJ DNA Engine (PTC 200, MJ Research) configured with a heated lid. PCR products for each primer set were visualized on 3% agarose gels stained with ethidium bromide and photographed. Random samples amplified with both the

P2/P8 and 2550F/2718R protocols were electrophoresed on 6% polyacrylamide gel to ensure adequate resolution was obtained with agarose.

Statistical analyses-Morphological characters were tested for differences between adult males and females and also juvenile males and females with analysis of variance (ANOVA; Zar 1996). The main factors in the models were gender and plot. Mass, wing chord, bill width, bill length, and tarsus length were the dependent variables. Discriminant analyses were also performed to assign adult and juvenile individuals into gender classes (DISCRIM; SAS Institute Inc. 1999). Wing chord, bill width, and bill length were included as criterion variables in the discriminant analysis. Chi-square analysis was performed to test if the observed offspring sex ratios differed from a 50:50 ratio. All analyses were conducted using the SAS software package (SAS Institute Inc. 1999). Differences were considered significant at $\alpha = 0.05$.

RESULTS

I was able to determine gender in all 211 individuals (162 adults, 49 juvenile/non-breeders) sampled in 2002 with the 2550F/2718R primer set and 3% agarose gel electrophoresis (Fig 2A; Fridolfsson and Ellegren 1999). Product was amplified with the P2/P8 primer set, however, the fragments were largely indistinguishable on 3% agarose (Fig 2B) or on 6% acrylamide gel. For adults, gender data collected in the field using breeding characteristics and behaviors were 100% consistent with the DNA analyses.

I assigned gender in 34 male and 15 female juvenile individuals that did not display sex-linked traits or behaviors in the field. Of the 49 juveniles sampled, 44 were from 17 distinct groups containing two or more siblings (Chapter 3). The ratio of male to female offspring among the sibling groups was 1.75:1 (with 28 males and 16 females), and was not significantly different from a 50:50 sex ratio (χ^2=1.67, d.f.=1, P=0.20).

Tables 1 and 2 summarize all morphological characters measured in 2001 and 2002. For adults, wing chord (F=40.5, d.f.=1, P<0.001) and bill width (F=32.2, d.f.=5, P<0.001) differed by gender; mass (F=1.73, d.f.=1, P=0.19), bill length (F=2.80, d.f.=1, P=0.10), and tarsus length (F=0.19, d.f.=1, P=0.67) did not differ. No morphological differences were detected between

the juvenile gender groups (mass: F=1.38, d.f.=1, P=0.25, wing chord: F=2.06, d.f.=1, P=0.16, bill length: F=0.37, d.f.=1, P=0.55, and tarsus length: F=0.01, d.f.=1, P=0.93).

Discriminant function analysis performed with bill width, bill length and wing chord variables correctly classified 87.1% of adult females and 77.5% of adult males to the gender groups previously determined with DNA (Table 3) and breeding characteristics. Results obtained from discriminant analyses of juvenile gender classes indicated that 73.3% of females and 67.7% males were correctly assigned to their respective groups (Table 4).

DISCUSSION

Until recently, few options were available to accurately identify gender in monomorphic species such as the Grasshopper Sparrow. Gender of adult individuals displaying territorial behavior and external sexual characters was reliably identified in all birds examined in the field. Observed gender differences in adult wing chord and bill width may be used in conjunction with the results obtained from discriminant analyses to increase gender determination efficiency in the field. Several other studies report comparable morphological data on the four Grasshopper Sparrow subspecies that breed in the United States (Table 5), however none examined gender differences with statistical methods. Mass and wing chord measurements reported for male and female Grasshopper Sparrows (*A. s. pratensis*) (Crossman 1989, Vickery 1996) in Connecticut and Maine are similar to both genders in West Virginia. Crossman (1989) reported bill length in Connecticut to be greater in both genders, while tarsus length estimates were greater in West Virginia.

Gender determination of individuals not displaying secondary sexual characteristics and behaviors is still problematic and cannot be resolved with the levels of precision and accuracy afforded with traditional morphological and behavioral cues. Significant differences in morphometrics between juveniles and adults were not surprising because a large percentage (86%) of the juveniles sampled in this study were 5-6 day old nestlings and recent fledglings not yet fully developed. Because of the age and development influences on morphological variation, gender could not be accurately assigned in juveniles using these characters.

Gender assignment data obtained for adult Grasshopper Sparrows by application of the 2550F/2718R primers (Fridolfsson and Ellegren 1999) was in 100% agreement with

morphological data collected in the field. Gender assignment with this primer set also proved an excellent alternative to the more invasive procedures normally required assign to gender in juvenile individuals. The limited success with the P2/P8 primers may be due to the fact that I did not cleave my PCR products with the *Hae*III restriction enzyme prior to electrophoresis (Boutette et al. 2002). This restriction site is reported to be highly conserved in the male *CHD-Z* gene in many species, but absent on the female *CHD-W* gene (Boutette et al. 2002).

The male to female sex ratio of the offspring examined in this study is slightly skewed (64:36 male to female) but was not statistically different from a 50:50 sex ratio. Eleven of the 17 broods were constructed using parentage and kinship analyses of microsatellite data (Chapter 3), and may contain individuals from multiple broods. Future research is required to generate large multiple year samples consisting of offspring from confirmed clutches. These data may be useful in gaining a better understanding of selective pressures influencing Grasshopper Sparrow natal philopatry, dispersal timing, and dispersal distance (Gowaty 1993)

The PCR techniques used in this study were found to be efficient, relatively inexpensive, and less invasive than traditional procedures. These methods do not require extensive molecular training and may be performed in many modestly equipped laboratories. Because the level of accuracy is high, data obtained from these techniques may be instrumental in providing answers to questions targeting dispersal mechanisms and winter assemblage composition in monomorphic species.

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Table 1. Mean and standard error (SE) mass and wing chord of Grasshopper Sparrows captured on mountaintop mine / valley fill complexes in southern West Virginia, 2001-2002.

		Mass (g)			Wing Chord (mm)		
Gender	N	Mean	SE	Range	Mean	SE	Range
Juvenile	79	14.3	0.22	9.00-18.0	47.6	1.56	35.5-59.5
male[a]	34	14.7	0.30	11.0-18.0	51.5	1.51	34.0-64.0
female[a]	15	14.0	0.60	27.0-59.0	47.3	2.69	27.0-59.0
Adult							
male	271	17.3	0.07	15.0-20.0	62.5	0.17	57.5-67.0
female	65	17.3	0.26	14.5-22.0	59.3	0.19	57.0-62.0

[a]Gender could be determined only for juveniles captured in 2002.

Table 2. Mean number and standard error (SE) and range of tarsus length, bill width, and bill length of Grasshopper Sparrows captured on mountaintop mine / valley fill complexes in southern West Virginia, 2002.

		Tarsus Length (mm)			Bill Width (mm)			Bill Length (mm)		
Gender	N	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Juvenile										
male	34	23.6	0.14	22-25	5.27	0.15	3.5-6.3	6.39	0.25	4.0-8.1
female	15	23.6	0.21	22-25	5.15	0.29	3.3-6.4	6.09	0.43	3.5-8.0
Adult										
male	131	24.7	0.12	19-27	6.28	0.03	3.5-6.8	8.06	0.03	7.1-8.9
female	31	24.7	0.12	23-25	6.03	0.05	5.5-6.5	7.90	0.06	7.3-8.7

Table 3. Classification results for adult male and female gender groups based on parametric quadratic discriminant function and error rates based on cross validation. Wing chord, bill width, and bill length were included as criterion variables in the discriminant analysis. Values are the number of observations assigned in each group (N) and the percent classified into each group (%).

Gender		Female	Male	Total
Female	N	27	4	31
	%	87.1	12.9	100.0
Male	N	29	100	129
	%	22.5	77.5	100.0
Total	N	56	104	160
	%	35.0	65.0	100.0
Priors	%	0.50	0.50	
Error Count				
Rate	%	0.13	0.22	0.18
Priors	%	0.50	0.50	

Table 4. Classification results for juvenile male and female gender groups based on parametric quadratic discriminant function and error rates based on cross validation. Wing chord, bill width, and bill length were included as criterion variables in the discriminant analysis. Values are the number of observations assigned in each group (N) and the percent classified into each group (%).

Gender		Female	Male	Total
Female	N	11	4	15
	%	73.3	26.7	100.0
Male	N	11	23	34
	%	32.4	67.6	100.0
Total	N	22	27	49
	%	44.9	55.1	100.0
Priors	%	0.50	0.50	
Error Count				
Rate	%	0.27	0.32	0.30
Priors	%	0.50	0.50	

Table 5. Comparison of Grasshopper Sparrow morphometrics of adult individuals sampled on reclaimed MTMVF complexes in southern West Virginia with those of previous studies.

Subspecies	N	Gender	Mass	Wing Chord	Bill Length	Tarsus Length	State	Source
A. s. floridanus	25	M	17.77 ± 0.20	60.74 ± 0.25			FL	Delaney et al. 1994
A. s. floridanus	5	F	18.38 ± 0.39	57.88 ± 0.30			FL	Delaney et al. 1994
A. s. perpallidus	16	M	16.80 ± 1.30	61.70 ± 1.80			SD	Collier 1994
A. s. perpallidus	6	F	17.00 ± 0.70	57.60 ± 1.60			SD	Collier 1994
A. s. ammolegus	60	M, F	17.00 ± 2.75				AZ	Dunning 1992
A. s. pratensis	8	M	17.82 ± 0.26	62.00 ± 0.33	11.93 ± 0.14	19.68 ± 0.33	CT	Crossman 1989
A. s. pratensis	1	F	18.75 ± 0.00	59.00 ± 0.00	10.90 ± 0.00	20.00 ± 0.00	CT	Crossman 1989
A. s. pra tensis	42	M	17.34 ± 0.17	59.80 ± 0.23			ME	Vickery 1996
A. s. pratensis	271	M	17.30 ± 0.07	62.50 ± 0.17	7.90 ± 0.06	24.70 ± 0.12	WV	This Study
A. s. pratensis	65	F	17.30 ± 0.26	59.30 ± 0.19	7.76 ± 0.16	24.70 ± 0.12	WV	This Study

Figure 1. Locations of study sites in southern West Virginia. Two fixed sampling plots were established on each mine complex and one plot was surveyed on the Mud River Wildlife Management Area.

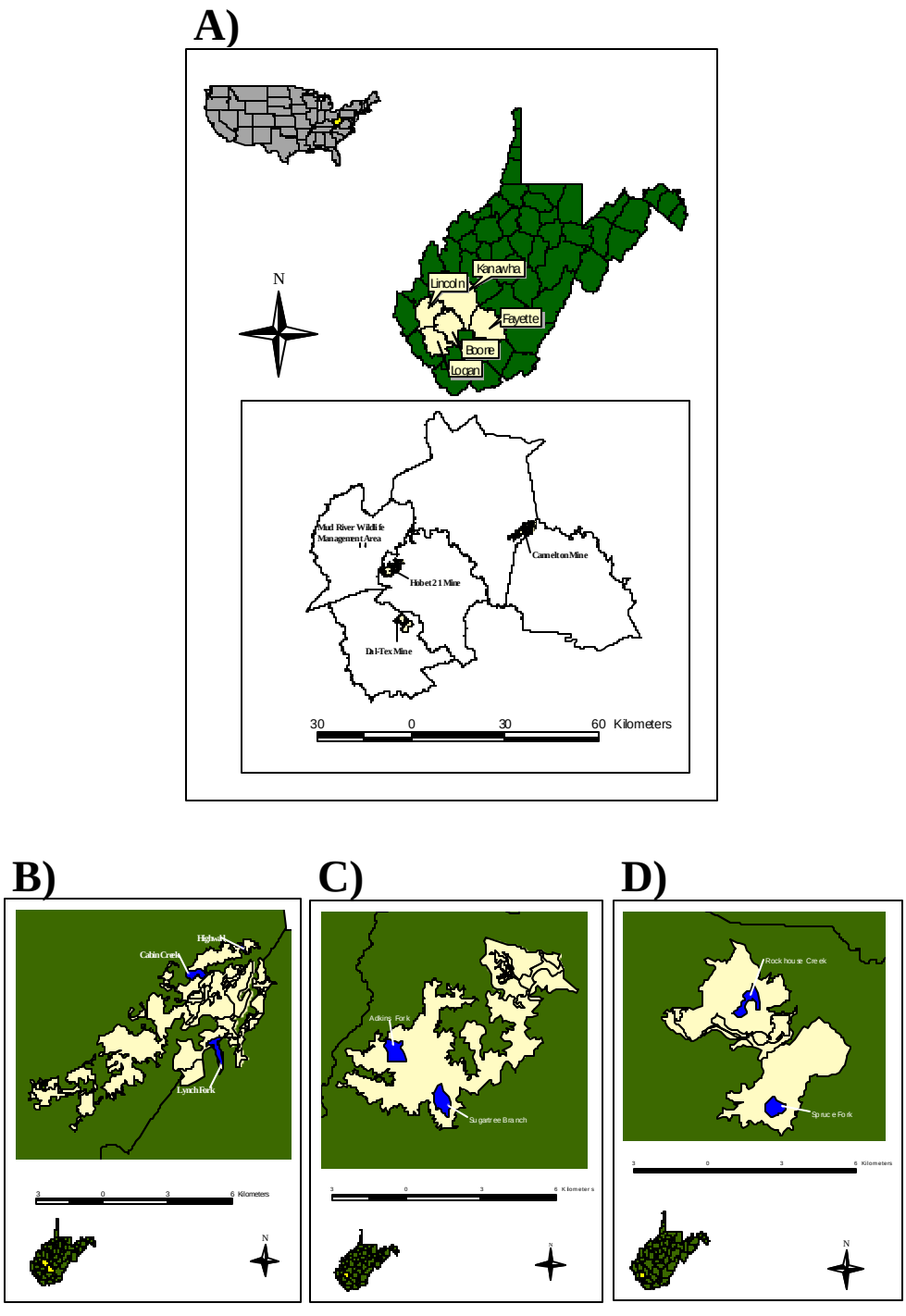
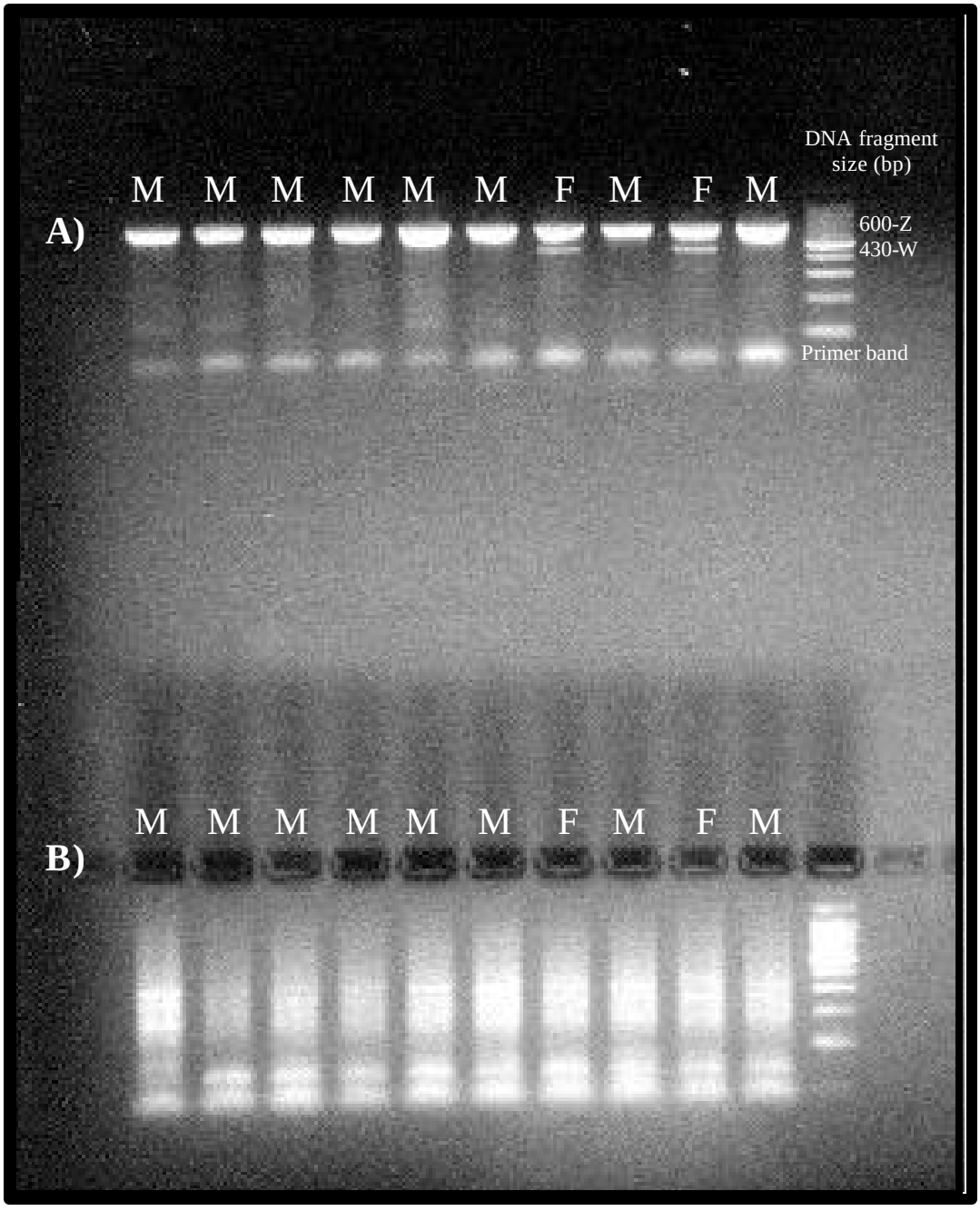


Figure 2. Gender typing of Grasshopper Sparrows using PCR methods. A) 3% agarose gel showing products amplified with the Fridolfsson and Ellegren 2550F/2718R primers; B) 3% agarose gel showing products amplified with the Griffiths et al. (1998) P2/P8 primer set. The same individuals were surveyed with both primer sets.



APPENDIX A

STUDY AREA AND MICROHABITAT VARIABLE DESCRIPTIONS

Table A. 1. Description of microhabitat variables measured at mined and non-mined subplots in southern West Virginia, 2001-2002.

Variable	Description and sampling method
Slope Aspect	Compass direction measured along gradient of slope at the plot or nest center.
Slope	Percentage of vertical rise over horizontal distance at the plot center.
Overhead Cover (%)	Percent overhead vegetation cover based on one ocular estimate measured at 1m above nest.
Side Cover (%)	Percentage of side cover based on the average of four ocular estimates, one from each side measured at 1m from nest.
Distance to Minor Edge (m)	Estimated distance to nearest minor road edge (m).
% Green	Percent coverage of live green vegetation ocularly estimated in a 11.3-m radius sampling subplot.
Ground Cover	Percent ground cover estimates were based on five ocular readings on each subplot every 2.26 m along four, 11.3-m transects that intersected at the center of the subplot.
Grass	Percent coverage of grass species.
Forb	Percent coverage of forb species.
Shrub	Percent coverage of woody shrub species.
Litter	Percent coverage of litter (dried grasses and forbs).
Wood	Percent coverage of woody debris (dead woody material).
Bare ground	Percent coverage of bare ground.
Moss	Percent coverage of moss species.
Water	Percent coverage of water.
Robel Pole Index (dm)	
Plot center	Average of four Robel height measurements at center of a five-meter radius plot.
1m	Average of four Robel height measurements at 1m from center of a five-meter radius plot.
3m	Average of four Robel height measurements at 3m from center of a five-meter radius plot.
5m	Average of four Robel height measurements at 5m from center of a five-meter radius plot.
Grass Height (dm)	Average grass height measured to the dm on 4, 11.3 m transects that intersected at the center of the subplot.
1m	Average of measurements at 1m from the plot center.
3m	Average of measurements at 3m from the plot center.
5m	Average of measurements at 5m from the plot center.
10m	Average of measurements at 10m from the plot center.
Litter depth (cm)	Average litter depth measured to the dm on 4, 11.3 m transects that intersected at the center of the subplot.
1m	Average of measurements at 1m from the plot center.
3m	Average of measurements at 3m from the plot center.

Table A. 1. Cont.

5m	Average of measurements at 5m from the plot center.
10m	Average of measurements at 10m from the plot center.
Tree Dist. from Nest (m)	Distance from nest or plot center in meters of largest tree within the 11.3-m radius sampling subplot.
Tree Height (m)	Height in meters of largest tree within the 11.3-m radius sampling subplot.
Tree Width (m)	Width in meters of the crown of the largest tree from the nest or subplot center, within the 11.3-m radius sampling subplot.
% Woody Cover on Plot	Percent coverage of live woody tree and shrub vegetation in the 11.3-m radius sampling subplot.
Shrub Dist. from Nest (m)	Distance in meters of largest shrub.
Shrub Height (m)	Height in meters of largest shrub.
Shrub Width (m)	Width in meters of largest shrub.
Nest substrate height (veg)(cm)	Height in cm of the vegetative growth of the primary nest substrate
Nest substrate height (repro)(cm)	Height in cm of the reproductive growth (seed head) of the primary nest substrate.
Nest Clump Area (cm^2)	Area of the contiguous vegetation clump used for nest construction.
Distance to foliage edge (cm)	Distance from nest opening to nearest bare ground.
Nest depth (cm)	Maximum nest depth measured from the top of the nest rim to nest center at time of fledge or failure.
Nest width (cm)	Maximum nest width measured inside of nest rim at time of fledge or failure.
Nest rim width (cm)	Maximum nest width measured at time of fledge or failure.
Nest rim height (cm)	Maximum nest rim height measured from ground to the bottom of the nest rim at time of fledge or failure.
Species occurrence	Vegetation species occurrence counts were based on five ocular readings on each subplot every 2.26 m along four, 11.3-m transects that intersected at the center of the subplot. The species visible in the crosshair of the sight-tube was identified and recorded. Species occurrence estimates for each sample plot was the sum of the sight tube measurements divided by 20.