Attachment 38

Comments on the Draft Plan submitted by Objectors on June 29, 2020, along with all attachments

Attachment 13

Species of Conservation Concern (SCC), Nantahala-Pisgah Forest Plan – July 2, 2015

Forest Service
Southern Region
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File Code: 1920
Date: July 2, 2015
Route To:

Subject: Species of Conservation Concern (SCC), Nantahala-Pisgah Forest Plan

To: Forest Supervisor, National Forests (NFs) in North Carolina

The 2012 Planning Rule requires that a Forest undergoing plan revisions to identify SCC. sec are at-risk species that are known to occur within the planning unit, but have "substantial concerns" about their ability to persist over the long-term in the plan area. As such, SCC represents a critical tool for Forest Service planners, biologists and managers, for conserving ecosystem diversity on the planning unit.

Under the 2012 Planning Rule, the Regional Forester identifies SCC for each planning unit, in consultation with the Forest Supervisor. The staff at the Regional Office has worked closely with the staff on the Forest to develop the list of SCC for the Nantahala-Pisgah NFs. Due to the high levels of biodiversity characteristic of the Nantahala-Pisgah NFs, many species had to be analyzed as potential SCC, and I appreciate the hard work and countless hours that the Forests, and their partners, have contributed to this process.

Pursuantto 36 CFR 219.7(c)(3), the enclosed list has been identified as 308 SCC for the Nantahala-Pisgah NFs. Please use this list to continue your work revising your Forest plan.

If you have any questions regarding the list of sec, the process for designating the sec, or the requirements for SCC under the 2012 Planning Rule, please contact Dr. Duke Rankin, Threatened and Endangered Species Program Manager at (404) 347-3981 or via email at draukin@fs.fed.us.

for Jerome Thomas
TONY TOOKE
Regional Forester

Enclosure

cc: Robert Trujillo, Peter Gaulke, Paul Arndt, Duke Rankin

Species of Conservation Concern

Nantahala and Pisgah National Forests

July 1, 2015

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Amphibian	Salamander	*Aneides aeneus*	green salamander	G3G4	S2
Amphibian	Salamander	*Desmognathus folkertsi*	dwarf black-bellied salamander	G2	S1
Amphibian	Salamander	*Desmognathus organi*	northern pygmy salamander	G3	S2
Amphibian	Salamander	*Eurycea junaluska*	Junaluska salamander	G3	S2
Amphibian	Salamander	*Plethodon aureolus*	Tellico salamander	G2G3	S2?
Amphibian	Salamander	*Plethodon chattahoochee*	Chattahoochee slimy salamander	G2G3Q	S1?
Amphibian	Salamander	*Plethodon cheoah*	Cheoah Bald salamander	G2	S1
Amphibian	Salamander	*Plethodon welleri*	Weller's salamander	G3	S2
Amphibian	Salamander Aquatic	*Cryptobranchus alleganiensis alleganiensis*	hellbender	G3G4	S3
Bird	Migratory bird	*Falco peregrinus*	peregrine falcon	G4	S1B,S2N
Bird	Migratory bird	*Setophaga cerulea*	cerulean warbler	G4	S2B
Bird	Migratory bird	*Vermivora chrystoptera*	golden-winged warbler	G4	S3B
Bird	Resident bird	*Haliaeetus leucocephalus*	bald eagle	G5	S3B,S3N
Bird	Resident bird	*Poecile atricapillus practica*	Southern Appalachian black-capped chickadee	G5T3	S3
Crustacean	Amphipod	*Stygobromus carolinensis*	Yancey sideswimmer	G1G2	S1
Crustacean	Crayfish	*Cambarus chaugaensis*	Chauga crayfish	G2	S2
Crustacean	Crayfish	*Cambarus georgiae*	Little Tennessee crayfish	G2	S2S3
Crustacean	Crayfish	*Cambarus parrishi*	Hiwassee headwaters crayfish	G2	S1
Fish	Fish	*Cottus carolinae*	banded sculpin	G5	S1
Fish	Fish	*Erimystax insignis eristigma*	southern blotched chub	G4TNR	S2
Fish	Fish	*Etheostoma acuticeps*	sharphead darter	G3	S1
Fish	Fish	*Etheostoma inscriptum*	turquoise darter	G4	S1
Fish	Fish	*Etheostoma vulneratum*	wounded darter	G3	S1
Fish	Fish	*Hybopsis rubrifrons*	rosyface chub	G4	S1
Fish	Fish	*Noturus flavus*	stonecat	G5	S1
Fish	Fish	*Percina caprodes*	logperch	G5	S1

Fish	Fish	*Percina squamata*	olive darter	G3	S2

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Plant Non-vascular	Lichen	*Heterodermia appalachiensis*	Appalachian Fringe Lichen	G2?	S1S2
Plant Non-vascular	Lichen	*Hypotrachyna oostingii*	Oosting's Square Britches	G1	S2
Plant Non-vascular	Lichen	*Hypotrachyna sinuosa*	A Foliose Lichen	G3G5	S1
Plant Non-vascular	Lichen	*Hypotrachyna virginica*	Virginia Loop Lichen	G1G2	S1S2
Plant Non-vascular	Lichen	*Leparia lanata*	Appalachian Dust Bunnies	G1	S1
Plant Non-vascular	Lichen	*Lobaria scrobiculata*	Textured Lungwort	G4	S2?
Plant Non-vascular	Lichen	*Melaniella stygia*	A Foliose Lichen	G4G5	S1S2
Plant Non-vascular	Lichen	*Micaraeopsis irriguata*	Irrigated Rock Lichen	G3	S2
Plant Non-vascular	Lichen	*Opegrapha moroziana*	Andy's Scribble Lichen	G1	S1
Plant Non-vascular	Lichen	*Pannaria conoplea*	A Foliose Lichen	G3G4	S1
Plant Non-vascular	Lichen	*Pertusaria appalachensis*	Appalachian Wart Lichen	G2?	S1?
Plant Non-vascular	Lichen	*Pilophorus fibula*	Appalachian Matchsticks	G1	S1
Plant Non-vascular	Lichen	*Punctelia reddenda*	Speckled Shield Lichen	G5	S2
Plant Non-vascular	Lichen	*Stereocaulon tennesseense*	Tennessee Twist	G2	S1
Plant Non-vascular	Lichen	*Sticta limbata*	Powdered Moon Lichen	G3G4	S1
Plant Non-vascular	Lichen	*Usnea angulata*	Old Man's Beard	G3G5	S1
Plant Non-vascular	Lichen	*Xanthoparmelia monticola*	A Rock-shield Lichen	G2?	S2?
Plant Non-vascular	Liverwort	*Acrobolbus ciliatus*	A Liverwort	G3?	S1
Plant Non-vascular	Liverwort	*Barbilophozia hatcheri*	A Liverwort	G5	S1
Plant Non-vascular	Liverwort	*Bazzania nudicaulis*	A Liverwort	G2G3	S2
Plant Non-vascular	Liverwort	*Cephaloziella spinicaulis*	A Liverwort	G3G4	S1
Plant Non-vascular	Liverwort	*Cheilolejeunea evansii*	A Liverwort	G1G2	S1
Plant Non-vascular	Liverwort	*Drepanolejeunea appalachiana*	A Liverwort	G2?	S1
Plant Non-vascular	Liverwort	*Frullania appalachiana*	A Liverwort	G1?	S1?
Plant Non-vascular	Liverwort	*Lejeunea blomquistii*	Blomquist Leafy Liverwort	G1G2	S1
Plant Non-vascular	Liverwort	*Leptoscyphus cunefolius*	Wedge Flapwort	G4G5	S2
Plant Non-vascular	Liverwort	*Lophocolea appalachiana*	A Liverwort	G1G2Q	S1
Plant Non-vascular	Liverwort	*Lophocolea muricata*	A Liverwort	G5	S1
Plant Non-vascular	Liverwort	*Mylia tayorii*	A Liverwort	G5	S1
Plant Non-vascular	Liverwort	*Plagiochila austinii*	A Liverwort	G3	S1S2
Plant Non-vascular	Liverwort	*Plagiochila corniculata*	A Liverwort	G4?	S2
Plant Non-vascular	Liverwort	*Plagiochila echinata*	A Liverwort	GNRT2	S1
Plant Non-vascular	Liverwort	*Plagiochila sharpii*	Sharp's Leafy Liverwort	G2G4	S2

Plant Non-vascular	Liverwort	*Plagiochila sullivantii var. sullivantii*	Sullivant's Leafy Liverwort	G2T2	S2

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Fish	Lamprey	*Lampetra appendix*	American brook lamprey	G4	S1
Insect	Butterfly	*Phyciodes batesii maconensis*	tawny crescent	G4T2T3	S2
Insect	Butterfly	*Speyeria aphrodite cullasaja*	Aphrodite fritillary	G5T1	S1?
Insect	Fly	*Eulonchus marialiciae*	Mary Alice's small-headed fly	G1G3	S1S3
Insect Aquatic	Caddisfly	*Rhyacophila amicis*	a caddisfly	G2	S2
Insect Aquatic	Dragonfly	*Ohpiogomphus edmundo*	Edmund's snaketail	G1G2	S1
Insect Aquatic	Dragonfly	*Ophiogomphus mainensis*	Maine snaketail	G5	S2
Mammal	Bat	*Corynorhinus rafinesquii rafinesquii*	Rafinesque's big-eared bat (mtn subspecies)	G3G4TNR	S2
Mammal	Bat	*Eptesicus fuscus*	big brown bat	G5	S5
Mammal	Bat	*Myotis leibii*	eastern small-footed bat (myotis)	G3	S3
Mammal	Bat	*Myotis lucifugus*	little brown bat (myotis)	G3	S4
Mammal	Bat	*Perimyotis subflavus*	tri-colored bat	G3	S5
Mammal	Rabbit	*Sylvilagus obscurus*	Appalachian cottontail	G4	S3
Mammal	Rodent	*Neotoma magister*	Appalachian woodrat	G3G4	S2S3
Mammal	Rodent	*Sorex palustris punctulatus*	southern water shrew	G5T3	S3
Mollusk	Freshwater mussel	*Alasmidonta varicosa*	brook floater	G3	S1
Mollusk	Freshwater mussel	*Alasmidonta viridis*	slippershell mussel	G4G5	S1
Mollusk	Freshwater mussel	*Fusconaia barnesiana*	Tennessee pigtoe	G2G3	S1
Mollusk	Freshwater mussel	*Pleurobema oviforme*	Tennessee clubshell	G2G3	S1
Mollusk	Freshwater mussel	*Villosa vanuxemensis*	mountain creekshell	G4	S1
Mollusk	Snail Aquatic	*Elimia christyi*	Christy's elimia	G2	S1
Mollusk	Snail Terrestrial	*Glyphyalinia clingmani*	fragile glyph	G1	S1
Mollusk	Snail Terrestrial	*Helicodiscus bonamicus*	spiral coil	G1	S1
Mollusk	Snail Terrestrial	*Helicodiscus triodus*	talus coil	G2	S1?
Mollusk	Snail Terrestrial	*Inflectarius subpalliatus*	velvet covert	G2	S2
Mollusk	Snail Terrestrial	*Paravitrea andrewsae*	high mountain supercoil	G2	S2
Mollusk	Snail Terrestrial	*Paravitrea ternaria*	sculpted supercoil	G1G2	S1
Mollusk	Snail Terrestrial	*Paravitrea varidens*	Roan supercoil	G1G2	S1S2
Plant Non-vascular	Lichen	*Anzia americana*	A Foliose Lichen	G3G5	S1
Plant Non-vascular	Lichen	*Arthonia kermesina*	Hot Dots	G1	S1
Plant Non-vascular	Lichen	*Arthopyrenia betulicola*	Ol' Birch Spots	G1	S1
Plant Non-vascular	Lichen	*Arthopyrenia degelii*	Degelius Spots	G1	S1
Plant Non-vascular	Lichen	*Fellhanera hybrida*	Piedmont Crustose Lichen	G2?	S1
Plant Non-vascular	Lichen	*Graphis sterlingiana*	Sterling Lips	G1	S1

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Plant Non-vascular	Liverwort	*Porella wataugensis*	A Liverwort	G1G2Q	S1
Plant Non-vascular	Liverwort	*Ptilidium ciliare*	A Liverwort	G5	S1
Plant Non-vascular	Liverwort	*Radula voluta*	A Liverwort	G3	S1
Plant Non-vascular	Liverwort	*Riccardia jugata*	A Liverwort	G1G2	S1?
Plant Non-vascular	Liverwort	*Sphenolobopsis pearsonii*	A Liverwort	G2?	S2
Plant Non-vascular	Moss	*Bartramidula wilsonii*	Dwarf Apple Moss	G4?	S1
Plant Non-vascular	Moss	*Brachydontium trichodes*	Peak Moss	G2G4	S1
Plant Non-vascular	Moss	*Brachythecium rotaeanum*	Rota's Feather Moss	G3G4	S1
Plant Non-vascular	Moss	*Bryoerythrophyllum ferruginascens*	A Moss	G4	S1
Plant Non-vascular	Moss	*Bryoxiphium norvegicum*	Sword Moss	G3G4	S1
Plant Non-vascular	Moss	*Campylopus atrovirens var. atrovirens*	Black Fish Hook Moss	G4G5TNR	S1?
Plant Non-vascular	Moss	*Dichodontium pellucidum*	A Moss	G4G5	S2
Plant Non-vascular	Moss	*Dicranum undulatum*	Bog Broom-moss	G5	S1
Plant Non-vascular	Moss	*Ditrichum ambiguum*	Ambiguous Ditrichium	G4?	S1
Plant Non-vascular	Moss	*Ditrichum rhynchostegium*	Golden Tread Moss	G3G5	S1?
Plant Non-vascular	Moss	*Encalypta procera*	Extinguisher Moss	G4G5	S1
Plant Non-vascular	Moss	*Entodon compressus*	Ftattened Entodon	G4	S1
Plant Non-vascular	Moss	*Entodon sullivantii*	Sullivant's Entodon	G3G4	S2
Plant Non-vascular	Moss	*Fissidens appalachensis*	Appalachian Pocket Moss	G2G3	S2S3
Plant Non-vascular	Moss	*Fontanalis sphagnifolia*	A Water Moss	G3G5	S1?
Plant Non-vascular	Moss	*Herzogiella turfacea*	Flat Stump Moss	G4G5	S1?
Plant Non-vascular	Moss	*Homalia trichomanoides*	Lime Homalia	G5	S1
Plant Non-vascular	Moss	*Homaliadelphus sharpii*	Sharp's Homaliadelphus	G3?	S1
Plant Non-vascular	Moss	*Leptodontium excelsum*	Grandfather Mountain Leptodontium	G2	S1
Plant Non-vascular	Moss	*Leptohymenium sharpii*	Mount Leconte Moss	G1	S1
Plant Non-vascular	Moss	*Palamocladium leskeoides*	Palamocladium	G3G5	S1
Plant Non-vascular	Moss	*Pilosium chlorophyllum*	A Moss	GNR	S1?
Plant Non-vascular	Moss	*Plagiomnium carolinianum*	Carolina Star-moss	G3	S2
Plant Non-vascular	Moss	*Plagiomnium ellipticum*	Marsh Magnificent Moss	G5	S1?
Plant Non-vascular	Moss	*Plagiomnium rostratum*	Long-beaked Thread Moss	G5	S1?
Plant Non-vascular	Moss	*Platyhypnidium pringlei*	Pringle's Eurhynchium	G2G3	S1
Plant Non-vascular	Moss	*Pogonatum dentatum*	Hair-like Hair-cap	G3G5	S1?
Plant Non-vascular	Moss	*Pohlia lescuriana*	Spherical Bulb Nodding Moss	G4?	S1?
Plant Non-vascular	Moss	*Pohlia rabunbaldensis*	A Moss	G1	SNR

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Plant Non-vascular	Moss	*Polytrichastrum alpinum*	Alpine Hair Moss	G4G5	S1?
Plant Non-vascular	Moss	*Racomitrium aciculare*	Dark Mountain Fringe Moss	G3G5	S1
Plant Non-vascular	Moss	*Rhabdoweisia crenulata*	Himalayan Ribbed-weissia	G3G4	S1
Plant Non-vascular	Moss	*Rhytidiadelphus subpinnatus*	A Moss	GU	S1?
Plant Non-vascular	Moss	*Rhytidium rugosum*	Golden Tundra-moss	G5	S2
Plant Non-vascular	Moss	*Schlotheimia lancifolia*	Highlands Moss	G2	S1
Plant Non-vascular	Moss	*Scopelophila cataractae*	Agoyan Cataract Moss	G3	S1
Plant Non-vascular	Moss	*Scopelophila ligulata*	Copper Moss	G5?	S1
Plant Non-vascular	Moss	*Sphagnum angustifolium*	Narrowleaf Peatmoss	G5	S1
Plant Non-vascular	Moss	*Sphagnum capillifolium*	Northern Peatmoss	G5	S1
Plant Non-vascular	Moss	*Sphagnum flexuosum*	Flexuous Peatmoss	G5Q	S1
Plant Non-vascular	Moss	*Sphagnum pylaesii*	Simple Peatmoss	G4	S1
Plant Non-vascular	Moss	*Sphagnum russowii*	Russow's Peatmoss	G5	S1
Plant Non-vascular	Moss	*Sphagnum squarrosum*	Squarrose Peatmoss	G5	S1
Plant Non-vascular	Moss	*Sphagnum tenellum*	Delicate Peatmoss	G5	S1
Plant Non-vascular	Moss	*Taxiphyllum alternans*	Japanese Yew-moss	G3?	S1
Plant Non-vascular	Moss	*Tortula ammonsiana*	Ammon's Tortula	G1G3	S1
Plant Non-vascular	Moss	*Warnstorfia fluitans*	Floating Sickle-moss	G5	S1
Plant Vascular	Clubmoss	*Huperzia porophila*	Rock Fir Clubmoss	G4	S2
Plant Vascular	Clubmoss	*Lycopodiella inundata*	Bog Clubmoss	G5	S1
Plant Vascular	Fern	*Asplenium bradleyi*	Bradley's Spleenwort	G4	S2
Plant Vascular	Fern	*Asplenium monanthes*	Single-Sorus Spleenwort	G4	S1
Plant Vascular	Fern	*Asplenium pinnatifidum*	Lobed Spleenwort	G4	S1
Plant Vascular	Fern	*Asplenium ruta-muraria*	Wall-rue	G5	S1
Plant Vascular	Fern	*Botrychium lanceolatum var. angustisegmentum*	Lance-leaf Moonwort	G5TNR	S1
Plant Vascular	Fern	*Botrychium matricariifolium*	Daisy-leaf Moonwort	G5	S1
Plant Vascular	Fern	*Botrychium multifidum*	Leathery Grape Fern	G5	S1
Plant Vascular	Fern	*Botrychium oneidense*	Blunt-lobed Grape Fern	G4Q	S2
Plant Vascular	Fern	*Botrychium simplex var. simplex*	Least Moonwort	G5T5	S2
Plant Vascular	Fern	*Cheilanthes alabamensis*	Alabama Lip-fern	G4G5	S1
Plant Vascular	Fern	*Cystopteris fragilis*	Fragile Fern	G5	S1
Plant Vascular	Fern	*Hymenophyllum tayloriae*	Gorge Filmy Fern	G2	S1S2
Plant Vascular	Fern	*Juncus trifidus*	Highland Rush	G5	S1
Plant Vascular	Fern	*Micropolypodium nimbatum*	West Indian Dwarf Polypody	G4?	S1

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Plant Vascular	Fern	*Phegopteris connectilis*	Northern Beech Fern	G5	S2
Plant Vascular	Fern	*Trichomanes boschianum*	Appalachian Filmy-fern	G4	S1
Plant Vascular	Fern	*Trichomanes petersii*	Dwarf Filmy-fern	G4G5	S2
Plant Vascular	Fern	*Woodsia appalachiana*	Appalachian Cliff Fern	G4	S1
Plant Vascular	Flowering Plant	*Adlumia fungosa*	Climbing Fumitory	G4	S2
Plant Vascular	Flowering Plant	*Arabis glabra*	Tower Mustard	G5	S1
Plant Vascular	Flowering Plant	*Arabis patens*	Spreading Rockcress	G3G4	S1
Plant Vascular	Flowering Plant	*Arisaema triphyllum ssp. stewardsonii*	Bog Jack-in-the-Pulpit	G5T4	S1
Plant Vascular	Flowering Plant	*Campanula aparinoides*	Marsh Bellflower	G5	S2
Plant Vascular	Flowering Plant	*Cardamine clematitis*	Mountain Bittercress	G3	S2
Plant Vascular	Flowering Plant	*Cardamine rotundifolia*	Mountain Watercress	G4	S2
Plant Vascular	Flowering Plant	*Chamerion platyphyllum*	Purple Willowherb	G5	S1
Plant Vascular	Flowering Plant	*Chelone cuthbertii*	Cuthbert's Turtlehead	G3	S3?
Plant Vascular	Flowering Plant	*Chelone obliqua var. erwiniae*	Mountain Purple Turtlehead	G2T2T4Q	S1
Plant Vascular	Flowering Plant	*Coreopsis grandiflora var. grandiflora*	Large-flowered Tickseed	G5T4T5	S1
Plant Vascular	Flowering Plant	*Corydalis micrantha*	Slender Corydalis	G5	S1
Plant Vascular	Flowering Plant	*Crocanthemum bicknellii*	Plains Sunrose	G5	S1
Plant Vascular	Flowering Plant	*Crocanthemum propinquum*	Creeping Sunrose	G4	S1
Plant Vascular	Flowering Plant	*Croton monanthogynus*	Prarie-tea Croton	G4	S1
Plant Vascular	Flowering Plant	*Dalibarda repens*	Robin Runaway	G5	S2
Plant Vascular	Flowering Plant	*Delphinium exaltatum*	Tall Larkspur	G3	S2
Plant Vascular	Flowering Plant	*Dodecatheon meadia*	Eastern Shooting Star	G5T5	S2
Plant Vascular	Flowering Plant	*Draba ramosissima*	Branching Draba	G4	S2
Plant Vascular	Flowering Plant	*Echinacea purpurea*	Purple Coneflower	G4	S1
Plant Vascular	Flowering Plant	*Epilobium ciliatum*	Purpleleaf Willowherb	G5	S2
Plant Vascular	Flowering Plant	*Erigenia bulbosa*	Harbringer-of-spring	G5	S1
Plant Vascular	Flowering Plant	*Eupatorium incarnatum*	Pink Throughwort	G5	S2
Plant Vascular	Flowering Plant	*Euphorbia purpurea*	Glade Spurge	G3	S2
Plant Vascular	Flowering Plant	*Filipendula rubra*	Queen-of-the-Prairie	G4G5	S1
Plant Vascular	Flowering Plant	*Frasera caroliniensis*	Columbo	G5	S2S3
Plant Vascular	Flowering Plant	*Gentiana saponaria var. latidens*	Balsam Gentian	G5T2T3	S2S3
Plant Vascular	Flowering Plant	*Gentianopsis crinita*	Fringed Gentian	G5	S1
Plant Vascular	Flowering Plant	*Geum geniculatum*	Bent Avens	G1G2	S1S2
Plant Vascular	Flowering Plant	*Helenium brevifolium*	Littleleaf Sneezeweed	G3G4	S1

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Plant Vascular	Flowering Plant	*Heuchera longiflora*	Long-Flower Alumroot	G4	S2
Plant Vascular	Flowering Plant	*Heuchera pubescens*	Downy Alumroot	G4?	S1
Plant Vascular	Flowering Plant	*Hexastylis contracta*	Mountain Heartleaf	G3	S1
Plant Vascular	Flowering Plant	*Hexastylis rhombiformis*	French Broad Heartleaf	G2	S2
Plant Vascular	Flowering Plant	*Hydrastis canadensis*	Goldenseal	G4	S2
Plant Vascular	Flowering Plant	*Liatris squarrulosa*	Earle's Blazing Star	G4G5	S2
Plant Vascular	Flowering Plant	*Liatris turgida*	Shale-barren Blazing Star	G3	S1S2
Plant Vascular	Flowering Plant	*Lilium grayi*	Gray's Lily	G3	S3
Plant Vascular	Flowering Plant	*Lilium philadelphicum var. philadelphicum*	Wood Lily	G5T4T5	S2
Plant Vascular	Flowering Plant	*Lysimachia fraseri*	Fraser's Loosestrife	G3	S3
Plant Vascular	Flowering Plant	*Minuartia groenlandica*	Greenand Sandwort	G5	S2
Plant Vascular	Flowering Plant	*Monarda media*	Purple Bee-balm	G4?	S1?
Plant Vascular	Flowering Plant	*Monotropsis odorata*	Sweet Pinesap	G3	S3
Plant Vascular	Flowering Plant	*Oenothera perennis*	Perennial Sundrops	G5	S1
Plant Vascular	Flowering Plant	*Packera millefolium*	Divided-leaf Ragwort	G2	S2
Plant Vascular	Flowering Plant	*Packera paupercula var. appalachiana*	Appalachian Barrens Ragwort	G5	S1
Plant Vascular	Flowering Plant	*Packera schweinitziana*	Schweinitz's Ragwort	G5?	S2
Plant Vascular	Flowering Plant	*Packera serpenticola*	Serpentine Ragwort	N/A	N/A
Plant Vascular	Flowering Plant	*Parnassia grandifolia*	Large-leaved Grass-of-parnassus	G3	S2
Plant Vascular	Flowering Plant	*Pedicularis lanceolata*	Swamp Lousewort	G5	S1
Plant Vascular	Flowering Plant	*Phlox subulata*	Moss Pink	G5	S1
Plant Vascular	Flowering Plant	*Polygala senega*	Seneca Snakeroot	G4G5	S2
Plant Vascular	Flowering Plant	*Pycnanthemum curvipes*	Stone Mountain-mint	G3	S1
Plant Vascular	Flowering Plant	*Ranunculus fascicularis*	Early Buttercup	G5	S1
Plant Vascular	Flowering Plant	*Rubus idaeus ssp. strigosus*	Red Raspberry	G5T5	S2?
Plant Vascular	Flowering Plant	*Rudbeckia triloba var. pinnatiloba*	Pinnate-lobed Black-eyed Susan	G5T3	S1
Plant Vascular	Flowering Plant	*Ruellia purshiana*	Pursh's Wild-petunia	G3	S2
Plant Vascular	Flowering Plant	*Sarracenia purpurea var. montana*	Southern Appalachian Purple Pitcher Plant	G5T1T3	S2?
Plant Vascular	Flowering Plant	*Scutellaria saxatilis*	Rock Skullcap	G3	S1
Plant Vascular	Flowering Plant	*Sedum glaucophyllum*	Cliff Stonecrop	G4	S2
Plant Vascular	Flowering Plant	*Shortia galacifolia var. brevistyla*	Northern Oconee Bells	G2G3T2	S2
Plant Vascular	Flowering Plant	*Shortia galacifolia var. galacifolia*	Southern Oconee Bells	G2G3T2T3	S2
Plant Vascular	Flowering Plant	*Silene ovata*	Mountain Catchfly	G3	S3
Plant Vascular	Flowering Plant	*Solidago simulans*	Granite Dome Goldenrod	G2	S2

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Plant Vascular	Flowering Plant	*Solidago uliginosa*	Bog Goldenrod	G4G5	S1S2
Plant Vascular	Flowering Plant	*Solidago ulmifolia*	Elm-leaf Goldenrod	G5	S1?
Plant Vascular	Flowering Plant	*Sparganium emersum*	Greenfruit Bur-reed	G5	S1
Plant Vascular	Flowering Plant	*Spigelia marilandica*	Pink root	G4	S1
Plant Vascular	Flowering Plant	*Stachys clingmanii*	Clingman's Hedge-nettle	G2	S2
Plant Vascular	Flowering Plant	*Stachys cordata*	Heartleaf Hedge-nettle	G5?	S1
Plant Vascular	Flowering Plant	*Stenanthium leimanthoides*	Pinebarren Death-camas	G4Q	S1
Plant Vascular	Flowering Plant	*Streptopus amplexifolius*	White Mandarin	G5	S1
Plant Vascular	Flowering Plant	*Symphyotrichum laeve var. laeve*	Smooth Blue Aster	G5T5	S1
Plant Vascular	Flowering Plant	*Symphyotrichum oblongifolium*	Aromatic Aster	G5	S1
Plant Vascular	Flowering Plant	*Symphyotrichum rhiannon*	Rhiannon's Aster	G1	S1
Plant Vascular	Flowering Plant	*Symphyotrichum shortii*	Short's Aster	G5	S1
Plant Vascular	Flowering Plant	*Thalictrum macrostylum*	Small-leaved Meadowrue	G3G4	S2
Plant Vascular	Flowering Plant	*Thaspium pinnatifidum*	Mountain Thaspium	G2G3	S1
Plant Vascular	Flowering Plant	*Thermopsis fraxinifolia*	Ash-leaved Gloden-banner	G3?	S2?
Plant Vascular	Flowering Plant	*Thermopsis mollis*	Appalachian Gloden-banner	G3G4	S2
Plant Vascular	Flowering Plant	*Triantha glutinosa*	Sticky Bog Asphodel	G4G5	S1
Plant Vascular	Flowering Plant	*Trichostema brachiatum*	Glade Bluecurls	G5	S1
Plant Vascular	Flowering Plant	*Trientalis borealis*	Starflower	G5	S1
Plant Vascular	Flowering Plant	*Trillium discolor*	Mottled Trillium	G4	S1
Plant Vascular	Flowering Plant	*Trillium simile*	Sweet White Trillium	G3	S2
Plant Vascular	Flowering Plant	*Veronica americana*	American Speedwell	G5	S2
Plant Vascular	Flowering Plant	*Viola appalachiensis*	Appalachian Violet	G4	S2
Plant Vascular	Grass	*Bouteloua curtipendula var. curtpiendula*	Sideoats Grama Grass	G5T5	S1
Plant Vascular	Grass	*Bromus ciliatus*	Fringed Brome	G5	S1
Plant Vascular	Grass	*Calamagrostis canadensis var. canadensis*	Canada Reedgrass	G5T5	S1
Plant Vascular	Grass	*Calamagrostis porteri*	Porter's Reedgrass	G4	S1
Plant Vascular	Grass	*Deschampsia cespitosa ssp. glauca*	Tufted Hairgrass	G5T5	S1
Plant Vascular	Grass	*Diarrhena americana*	Eastern Beakgrass	G4?	S1
Plant Vascular	Grass	*Elymus trachycaulus ssp. trachycaulus*	Slender Wheatgrass	G5T5	S1
Plant Vascular	Grass	*Glyceria laxa*	Lax Mannagrass	G5	S1
Plant Vascular	Grass	*Glyceria nubigena*	Smoky Mountain Mannagrass	G2	S2
Plant Vascular	Grass	*Melica nitens*	Three-flowered Melica	G5	S1
Plant Vascular	Grass	*Muhlenbergia glomerata*	Bristly Muhly	G5	S1

Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Plant Vascular	Grass	*Poa palustris*	Swamp Bluegrass	G5	S1
Plant Vascular	Grass	*Poa saltuensis*	A Bluegrass	G5	S1
Plant Vascular	Grass	*Spartina pectinata*	Freshwater Cordgrass	G5	S1
Plant Vascular	Grass	*Sporobolus heterolepis*	Prairie Dropseed	G5	S1
Plant Vascular	Orchid	*Arethusa bulbosa*	Bog Rose	G4	S1
Plant Vascular	Orchid	*Cleistes bifaria*	Small Spreading Pogonia	G4?	S2
Plant Vascular	Orchid	*Corallorhiza wisteriana*	Spring Coral-root	G5	S2
Plant Vascular	Orchid	*Cypripedium parviflorum var. parviflorum*	Small Yellow Lady's-slipper	G5T3T5	S1S2
Plant Vascular	Orchid	*Hexalectris spicata*	Crested Coralroot	G5	S2
Plant Vascular	Orchid	*Liparis loeselii*	Fen Orchid	G5	S1
Plant Vascular	Orchid	*Platanthera flava var. herbiola*	Northern Green Orchid	G4T4Q	S1?
Plant Vascular	Orchid	*Platanthera grandiflora*	Large Purple-fringed Orchid	G5	S2
Plant Vascular	Orchid	*Platanthera peramoena*	Purple Fringeless Orchid	G5	S2
Plant Vascular	Sedge	*Carex baileyi*	Bailey's Sedge	G4	S2
Plant Vascular	Sedge	*Carex careyana*	Carey's Sedge	G4G5	S1
Plant Vascular	Sedge	*Carex cherokeensis*	Cherokee Sedge	G4G5	S1
Plant Vascular	Sedge	*Carex hitchcockiana*	Hitchcock's Sedge	G5	S1
Plant Vascular	Sedge	*Carex oligocarpa*	Rich-woods Sedge	G4	S1
Plant Vascular	Sedge	*Carex oligosperma*	Few-seeded Sedge	G5?	S1
Plant Vascular	Sedge	*Carex pedunculata*	Longstalk Sedge	G5	S2
Plant Vascular	Sedge	*Carex projecta*	Necklace Sedge	G5	S1
Plant Vascular	Sedge	*Carex purpurifera*	Purple Sedge	G4?	S2
Plant Vascular	Sedge	*Carex radfordii*	Radford's Sedge	G2	S1
Plant Vascular	Sedge	*Carex roanensis*	Roan Sedge	G2G3	S2
Plant Vascular	Sedge	*Carex utriculata*	Beaked Sedge	G5	S1
Plant Vascular	Sedge	*Rhynchospora alba*	Northern White Beaksedge	G5	S2
Plant Vascular	Shrub	*Alnus viridis ssp crispa*	Green Alder	G5T5	S1
Plant Vascular	Shrub	*Berberis canadensis*	American Barberry	G3	S2
Plant Vascular	Shrub	*Buckleya distichophylla*	Piratebush	G3	S2
Plant Vascular	Shrub	*Diervilla rivularis*	Riverbank Bush-honeysuckle	G3	S1
Plant Vascular	Shrub	*Fothergilla major*	Large Witch-alder	G3	S3
Plant Vascular	Shrub	*Lonicera canadensis*	American Fly-honeysuckle	G5	S2
Plant Vascular	Shrub	*Rhododendron cumberlandense*	Cumberland Azalea	G4?	S1
Plant Vascular	Shrub	*Rhododendron vaseyi*	Pink-shell Azalea	G3	S3

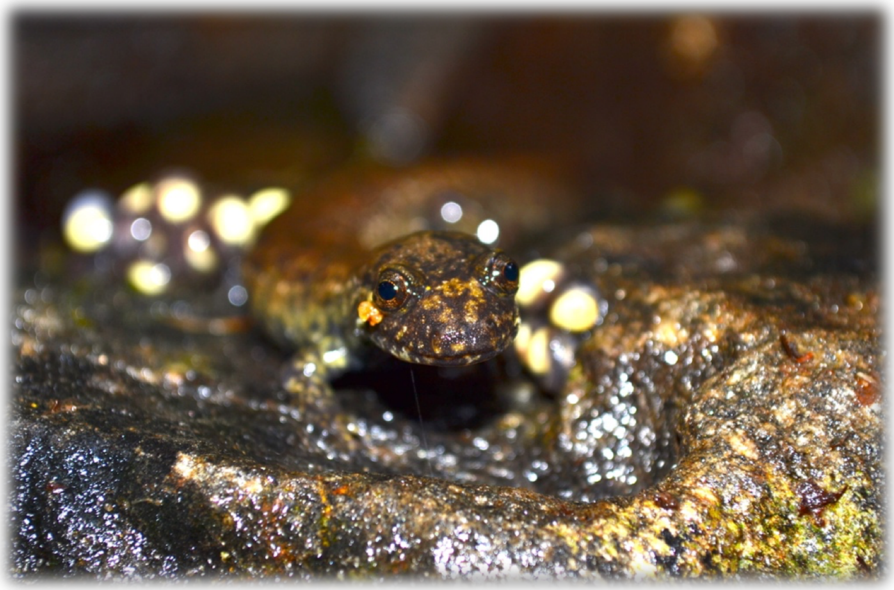
Taxonomic Group	**Taxonomic Subgroup**	**Species**	**Common Name**	NatureServe Rank	North Carolina Rank
Plant Vascular	Shrub	*Robinia hispida var fertilis*	Fruitful Locust	G5T1Q	S1
Plant Vascular	Shrub	*Robinia hispida var kelseyi*	Kelsey's Locust	G5T1	S1
Plant Vascular	Shrub	*Robinia viscosa var. hartwigii*	Hartwig's Locust	G3T2	S2
Plant Vascular	Shrub	*Robinia viscosa var. viscosa*	Clammy Locust	G3T3	S3
Plant Vascular	Shrub	*Stewartia ovata*	Mountain Camellia	G4	S2
Plant Vascular	Shrub	*Vaccinium macrocarpon*	Cranberry	G4	S2
Plant Vascular	Tree	*Betula cordifolia*	Mountain Paper Birch	G5	S1
Plant Vascular	Vine	*Celastrus scandens*	American Bittersweet	G5	S2?
Reptile	Turtle	*Glyptemys muhlenbergii*	bog turtle	G3	S2

n = 308

Attachment 14

An Analysis of Important Areas for Salamander Conservation and Connectivity in the Nantahala and Pisgah National Forests – Spring 2019

An Analysis of Important Areas for Salamander Conservation and Connectivity in the Nantahala and Pisgah National Forests



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A report submitted to The Southern Environmental Law Center and The Wilderness Society

Spring 2019

Introduction and Purpose

Global amphibian declines have been of concern to scientists, conservationists, and land managers since the 1980s (Stuart et al. 2004). The severity of amphibian declines has been shocking, with catastrophic losses occurring across the globe and in a wide variety of habitats (e.g. Sherman and Morton 1993, Drost and Fellers 1996, Pounds et al. 1997, Pounds and Crump 1994, Ron et al. 2003, Young et. al. 2001). More recent assessments have revealed that nearly half of all amphibians are threatened with extinction and nearly all species continue to decline (Stuart et al. 2004, Grant et al. 2016). These trends make amphibians among the most imperiled vertebrate groups in the world.

Within amphibians, there are also clear patterns that have emerged regarding threat level (Stuart et al. 2004, Nowakowski et al. 2018). Notably, salamanders as a group tend to be highly imperiled, with roughly 60% of species considered to be at risk (www. IUCN.org, Apodaca 2010). This is significant for the Southern Appalachians in general and for the Nantahala and Pisgah National Forests, as they are central to what has long been considered the world's hotspot of salamander diversity (Fig. 1). In fact, the region as a whole contains roughly one fifth of the world's salamander diversity and more families and genera than anywhere in the world. Meaning that not only does this area have an incredible diversity of salamanders, but also a high amount of "deep" or phylogenetic diversity in the region.

Unfortunately, the Southern Appalachians have not escaped the trend in worldwide amphibian declines. In fact, within the Pisgah and Nantahala National Forests, there are several species of concern (State listed species, IUCN priority species, US Forest Service (USFS) priority species, and species under review for listing under the Endangered Species Act) and a history of enigmatic declines for some species (e.g. Snyder 1983, Corser and Gaddy 1991, Snyder 1991, Petranka et al. 1993, Corser 2001).

It has also become clear that there is no single cause of amphibian declines, but rather the coalescence of several factors such as habitat loss, disease, climate change, and fragmentation. Consequently, the conservation and management of amphibians, and salamanders in particular, requires a comprehensive approach. While there is no panacea, the long-term persistence of amphibians is highly dependent on healthy metapopulations (Hanski and Gilpin 1991, Hanski and Ovaskainen 2000, Cushman 2006, Apodaca et al. 2012, Cushman et al. 2012). Functioning metapopulations tend to maintain genetic diversity and minimize the effects of inbreeding (Frankham et al. 2002, Apodaca 2010, Apodaca et al. 2012). Genetic diversity provides populations with the ability to adapt to changing conditions (climate fluctuations, disease, invasive species, etc.), and inbreeding diminishes genetic diversity and all-around population health and fitness (Frankham et al. 2002, Pauls et al. 2013). Just as amphibians depend on metapopulation health, metapopulations depend on available habitat patches and an intact and connected landscape.

With over a million acres in total area, the Nantahala and Pisgah National Forests provide the ideal situation to establish large and functioning metapopulations for several species of salamander in the world's center of diversity for them. Currently, salamanders are not factored into forest-wide management decisions. Nor is connectivity considered, even for priority species, in a systematic way. Here, we have attempted to create a tool that identifies USFS stands that are disproportionately important to salamander persistence, connectivity, and metapopulation function within the Nantahala and Pisgah National Forests. By managing the identified stands in a manner beneficial to salamanders, the USFS can help to assure that the Southern Appalachians maintain the rare and endemic salamander diversity found in the region.

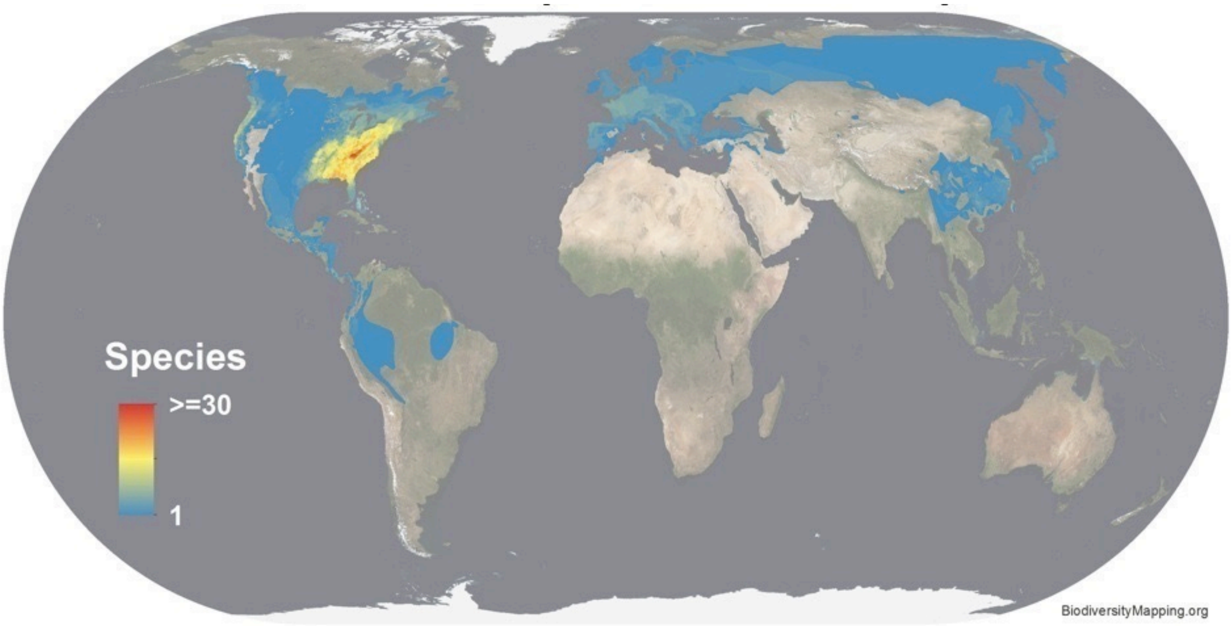


Figure 1. Worldwide salamander diversity.

Methods

Overall Approach

We began by creating environmental niche models (ENMs) for target species. In general, environmental niche models (ENMs) create a prediction of a species' geographic range by relating a species known locality data to environmental parameters. ENMs have been successfully integrated into a diverse set of ecological (e.g., Araújo & Williams, 2000; Ferrier et al., 2002; Mac Nally & Fleishman, 2004, Cunningham et al., 2009), evolutionary (e.g. Graham et al., 2004; Wiens & Graham, 2005; Rissler & Apodaca, 2007), and conservation (e.g. Ferrier, 2002; Raxworthy et al., 2003; Domínguez-Domínguez et al., 2006; Garcia, 2006; Rissler et al., 2006) studies. We then used a tiered threshold to identify 3 levels of habitat and climate space (sub optimal, adequate, and optimal) for each species. From these distribution models, we then created additive distribution models (Apodaca 2010) for each ecological group.

This approach allowed us to identify areas that were of high ecological value for several species without losing the biological reality of the individual species' niche. These maps also provided a basis for identifying core areas for additional analyses.

Running the Maxent Program

23 variables (19 climate variables and 4 habitat variables) were used for niche modeling in Maxent. GIS layers for climate variables were obtained from WorldClim (WorldClim Version 2 BioClimatic Variables bio30s 1970-2000, http://worldclim.org/version2). Climate variables used in the analysis are listed below.

- Annual Mean Temperature
- Mean Diurnal Range (Mean of monthly (max temp - min temp))
- Isothermality
- Temperature Seasonality
- Max Temperature of Warmest Month
- Min Temperature of Coldest Month
- Temperature Annual Range
- Mean Temperature of Wettest Quarter
- Mean Temperature of Driest Quarter
- Mean Temperature of Warmest Quarter

- Mean Temperature of Coldest Quarter
- Annual Precipitation
- Precipitation of Wettest Month
- Precipitation of Driest Month
- Precipitation Seasonality (Coefficient of Variation)
- Precipitation of Wettest Quarter
- Precipitation of Driest Quarter
- Precipitation of Warmest Quarter
- Precipitation of Coldest Quarter

Habitat variable GIS layers used were Canopy Cover (NLCD 2011 USFS Tree Canopy cartographic (CONUS), https://www.mrlc.gov/data?f%5B0%5D=category%3Acanopy), Stream Locations (USGS 24k Hydrography, https://www.usgs.gov/core-science-systems/ngp/national-hydrography/access-national-hydrography-products), Vegetation Cover (North Carolina Land Cover, http://www.basic.ncsu.edu/segap/), and Soil Moisture (Soil Survey Geographic Database (SSURGO)). Each layer was uploaded to ArcGIS Pro 2.2.4 where it was edited to be compatible with the Maxent program. The Stream Locations layer was converted via the Euclidean Distance tool to a layer displaying each cell's distance from a stream. Cell size for each raster was set to 100m.

Species location data was obtained from museums (through the GBIF portal), iNaturalist, and HerpMapper, with supplemental data points provided by local experts. The species included in the niche model were divided into 4 subgroups, listed below.

- Rock Outcrop Specialist Subgroup
    - *Aneides aeneus* (Sample Size 311)
- Streamside Subgroup
    - *Pseudotriton ruber* (Sample Size 72)
    - *Eurycea guttolineata* (Sample Size 20)
    - *Eurycea wilderae* (Sample Size 101)
    - *Eurycea cirrigera* (Sample Size 14)
- Woodland Subgroup
    - *Notophthalmus viridescens* (Sample Size 46)
    - *Plethodon yonahlossee* (Sample Size 34)

    - *Plethodon teyahalee* (Sample Size 13)
    - *Plethodon shermani* (Sample Size 20)
    - *Plethodon serratus* (Sample Size 13)
    - *Plethodon montanus* (Sample Size 33)
    - *Plethodon metcalfi* (Sample Size 42)
    - *Plethodon cylindraceus* (Sample Size 54)
    - *Plethodon cinereus* (Sample Size 19)
- High Elevation Subgroup
    - *Desmognathus organi* (Sample Size 11)
    - *Desmognathus wrighti* (Sample Size 33)
    - *Plethodon welleri* (Sample Size 12)

The variable GIS layers and species location data were then used to run a species niche model through Maxent. Each species was run as a separate model.

Processing Maxent Output Rasters

After the Maxent Program model had been run for each species, the output niche rasters were uploaded to ArcGIS Pro. The rasters were reclassified so that the highest value pixel had a score of 2, the second highest 1, and the rest 0. Each species' raster was then added together within their subgroup to create a subgroup-level Additive Distribution Model (ADM) map.

Within each subgroup, the ADM maps were used to create input data for the connectivity analysis tools in the Linkage Mapper Toolbox (version 2.0.0). Reclassifying the ADM raster to reverse the values created the "resistance raster", or a map whose cells have an attributed value reflecting the energetic cost, difficulty, or mortality risk of moving across that cell. The ADM map was also used to create a "core areas" layer, containing regions with the highest suitability score. The number of top values included in the core areas layer depended on the subgroup, with the Rock Outcrop Specialist Subgroup including the top 2 values, the Streamside Subgroup including the top 3, the Woodland Subgroup including the top 5, and the High Elevation Subgroup including the top 3. The number of values to include was determined based on the range of values in each subgroup. For example, the Rock Outcrop Specialist Subgroup's ADM values ranged from 0-2, but the Woodland Subgroup ranged from 0-13. The Core Area layer was aggregated to reduce the number of cores by combining independent, single- cell polygons within close proximity of each other.

Running the Linkage Mapper Toolbox

The Linkage Mapper Toolbox 2.0.0 was used to conduct connectivity analysis. The Linkage Pathways Tool was run in order to identify and map least-cost linkages between core areas. The maximum cost-weighted corridor distance was set to 70,000 meters to prevent extremely remote cores from being connected.

The Linkage Priority tool was then run to quantify the relative conservation priority of each linkage in a landscape. For CAV Calculations, the weight given to Resistance and Size was set to 0.33 and Area/Perimeter was set to 0.34 in order to make the sum 1.0.

The Core Centrality tool was run to calculate current flow centrality, a measure of how important a link or core area is for keeping the overall network connected.

The Barrier Mapper tool was run to detect important barriers that affect the quality and/or location of the corridors. The minimum detection radius, or the minimum search radius for moving window analysis, was set to 250 meters as this is half the minimum length of a strip of land that could be restored. The maximum detection radius, or maximum search radius for moving window analysis, was set to 1000 meters as this is half the maximum length of a strip of land that could be restored. The radius step value was set to 0 so that the program would only search for barriers at a single radius.

Lastly, the Pinchpoint Mapper was run to create current maps that identify and map pinch points (i.e. constrictions, a.k.a. bottlenecks or choke points) in least-cost corridors (Linkage Mapper 2.0.0 User Guide). The CWD Cutoff Distance, or cost-weighted corridor “width”, was set to 5,000 meters, a value selected based on information from chapter 2 of WHCWG (2010).

Results and Implications

For each subgroup, we feel that the most important models for salamander management within the Nantahala and Pisgah National Forests are the Core Areas Model (CAM), the Linkage Priority Model (LPM), and the Least Cost Path Model (LCP). Undoubtedly, the stands identified in the CAM (Figs. 2,5,8,9,14, and 15) are the top priority, as they show the areas that have the best current condition for each subgroup. By managing these areas at the stand level for salamander diversity, the USFS can efficiently maintain healthy metapopulations for the identified taxa. We would suggest that harvest in these areas is limited, as research has shown that most Southern Appalachian salamander communities can take a century or more to recover from unnatural disturbance regimes (Herbeck and Larsen 1999, Petranka 1999, Connette and Semlitsch 2013, Hocking et al. 2013).

The stands identified as high priority by the LPM (Figs 3, 6, 10, 11, 16, and 17) and LCP (Figs. 4, 7, 12, 13, 18, and 19) are important to maintain connectivity between the identified core areas. Connectivity is vital to the long-term survival of populations, metapopulations, and ultimately species. These models identify stands that are highly suitable and are high priority for linking populations (LPM) or that represent the biologically shortest path between identified core areas (LCP) that contains habitat that the species are able to disperse through. All of the species analyzed here, save one (*Notophthalmus viridescens),* are Plethodontid or lungless salamanders, which are notoriously poor dispersers (Dowling 1956, Jaeger and Forester 1993, Martin et al. 2016). For many of these species, migration will likely not occur across even relatively small swaths of unsuitable habitat. Therefore, maintaining continuous habitat within high priority stands is recommended. We suggest that timber harvest within these stands is limited to non-adjacent compartments and that streamside BMPs are strictly adhered to and road building/ staging areas are limited. We have provided several other useful tools in the appendix for identifying key areas to consider for salamander persistence and connectivity within the Nantahala and Pisgah National Forests.

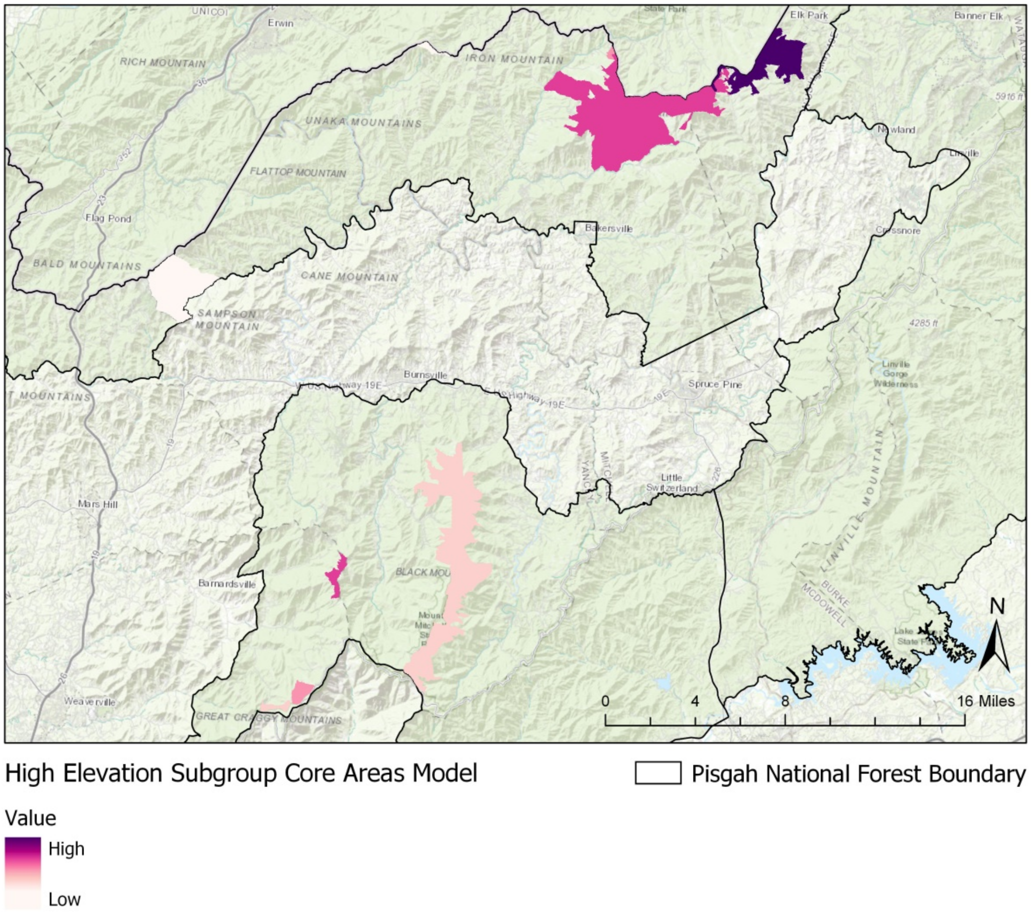


Figure 2. Map showing forest stands within the Pisgah National Forest containing core areas for the High Elevation Subgroup as determined by the Core Area Model. Stands containing core areas with a higher priority value are dark purple while stands containing core areas with a lower priority value are light pink.

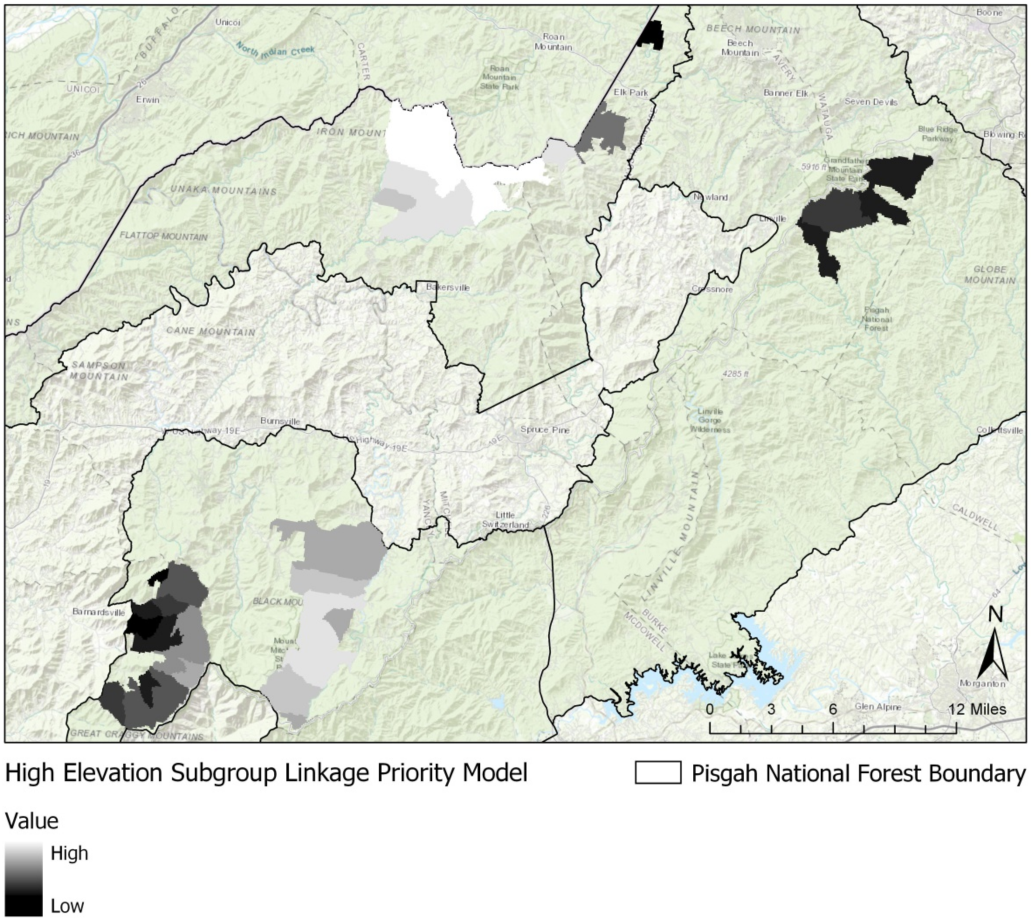


Figure 3. Map showing forest stands within the Pisgah National Forest containing linkages between cores for the High Elevation Subgroup as determined by the Linkage Priority Model. Stands containing linkages with a higher priority value are white while stands containing linkages with a lower priority value are black.

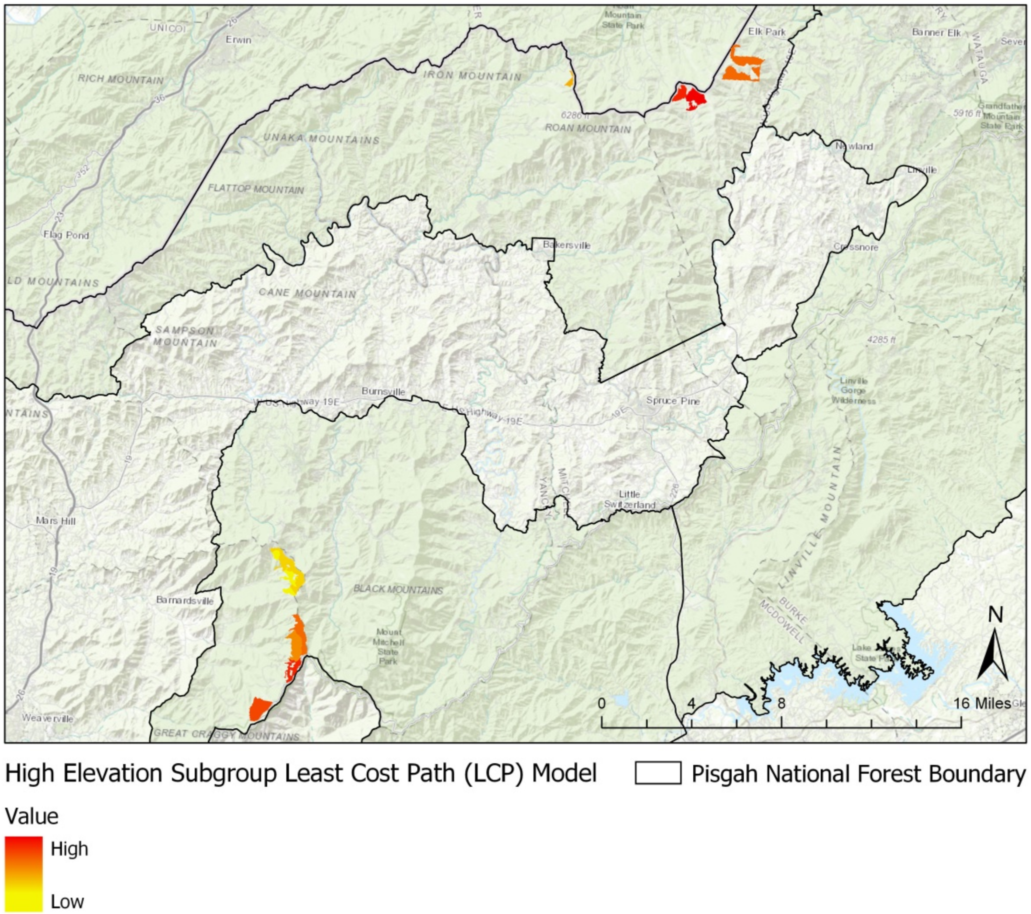


Figure 4. Map showing forest stands within the Pisgah National Forest containing least cost paths between cores for the High Elevation Subgroup as determined by the Least Cost Path (LCP) Model. Stands containing paths with a higher priority value are red while stands containing paths with a lower priority value are yellow.

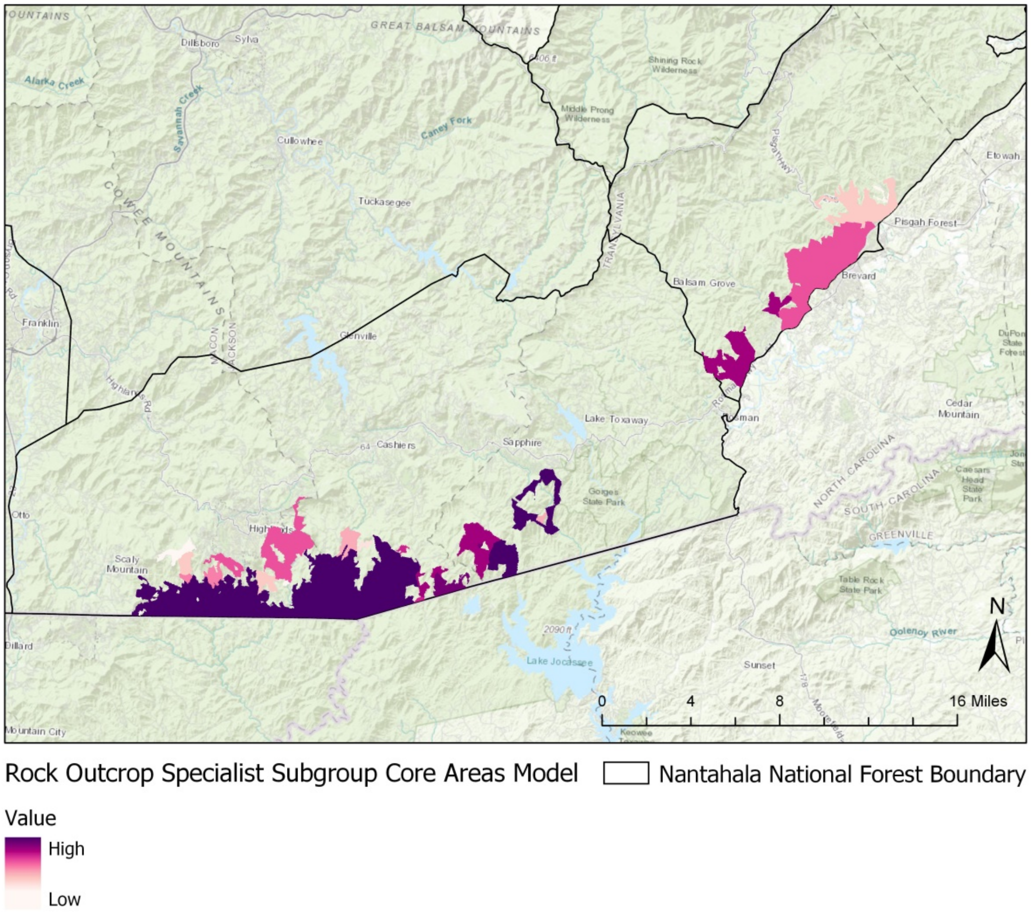


Figure 5. Map showing forest stands within the Nantahala National Forest containing core areas for the Rock Outcrop Specialist Subgroup as determined by the Core Area Model. Stands containing core areas with a higher priority value are dark purple while stands containing core areas with a lower priority value are light pink.

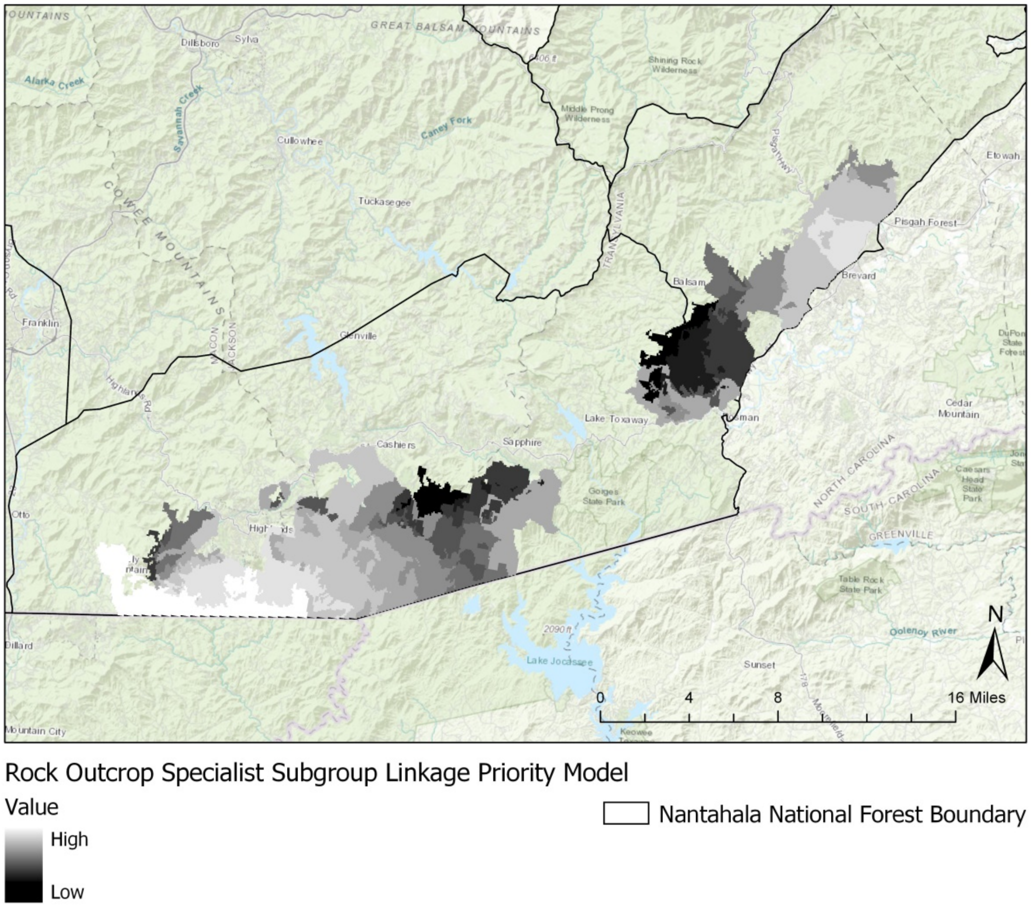


Figure 6. Map showing forest stands within the Nantahala National Forest containing linkages between cores for the Rock Outcrop Specialist Subgroup as determined by the Linkage Priority Model. Stands containing linkages with a higher priority value are white while stands containing linkages with a lower priority value are black.

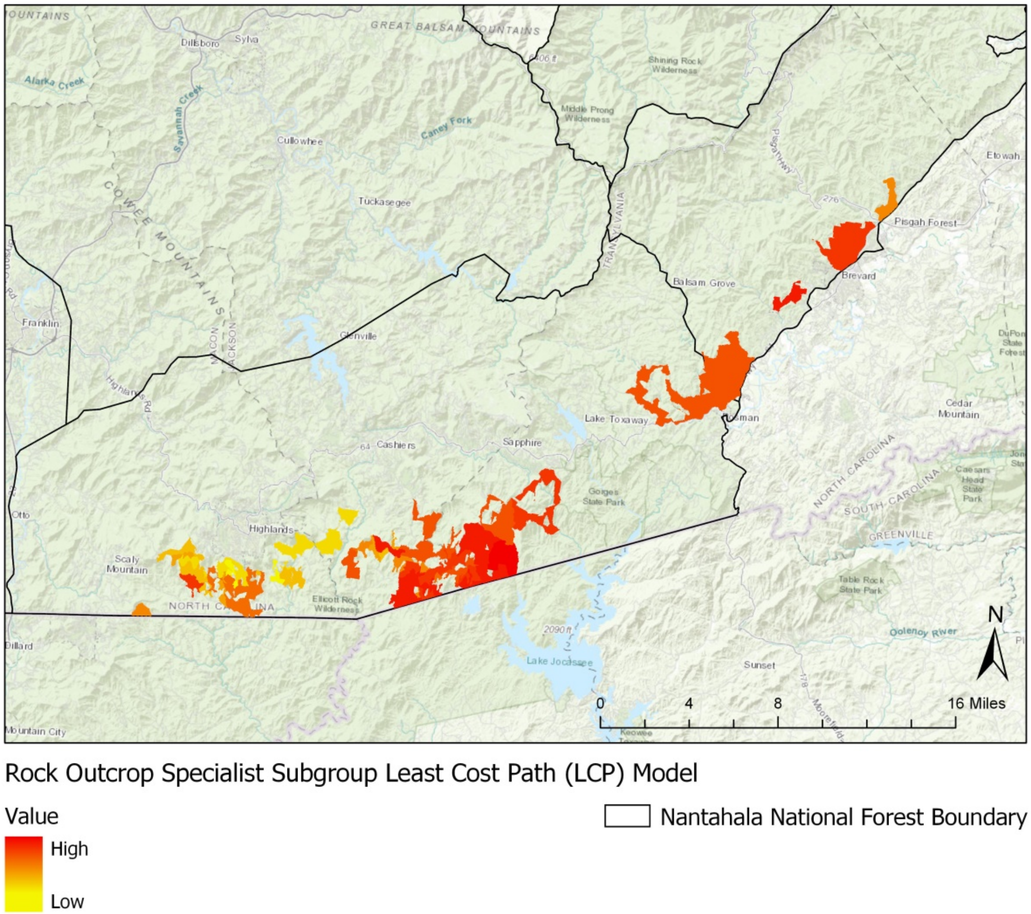


Figure 7. Map showing forest stands within the Nantahala National Forest containing least cost paths between cores for the Rock Outcrop Specialist Subgroup as determined by the Least Cost Path (LCP) Model. Stands containing paths with a higher priority value are red while stands containing paths with a lower priority value are yellow.

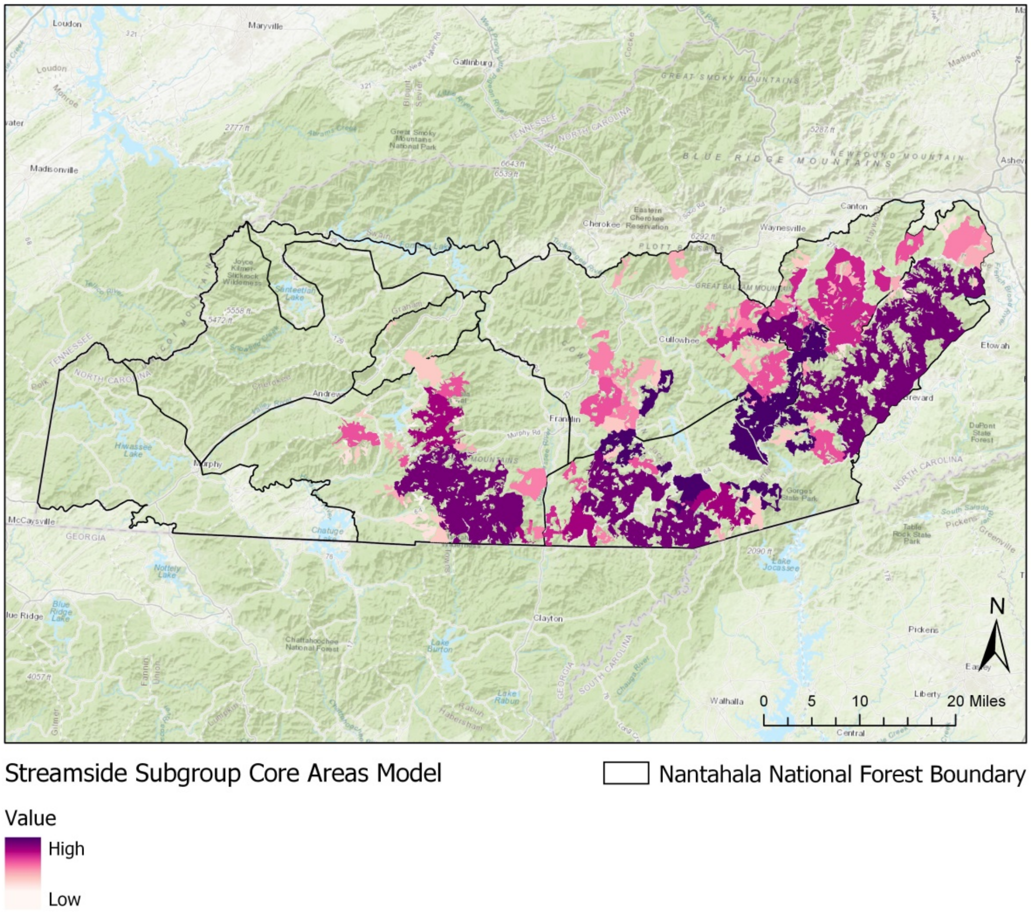


Figure 8. Map showing forest stands within the Nantahala National Forest containing core areas for the Streamside Subgroup as determined by the Core Area Model. Stands containing core areas with a higher priority value are dark purple while stands containing core areas with a lower priority value are light pink.

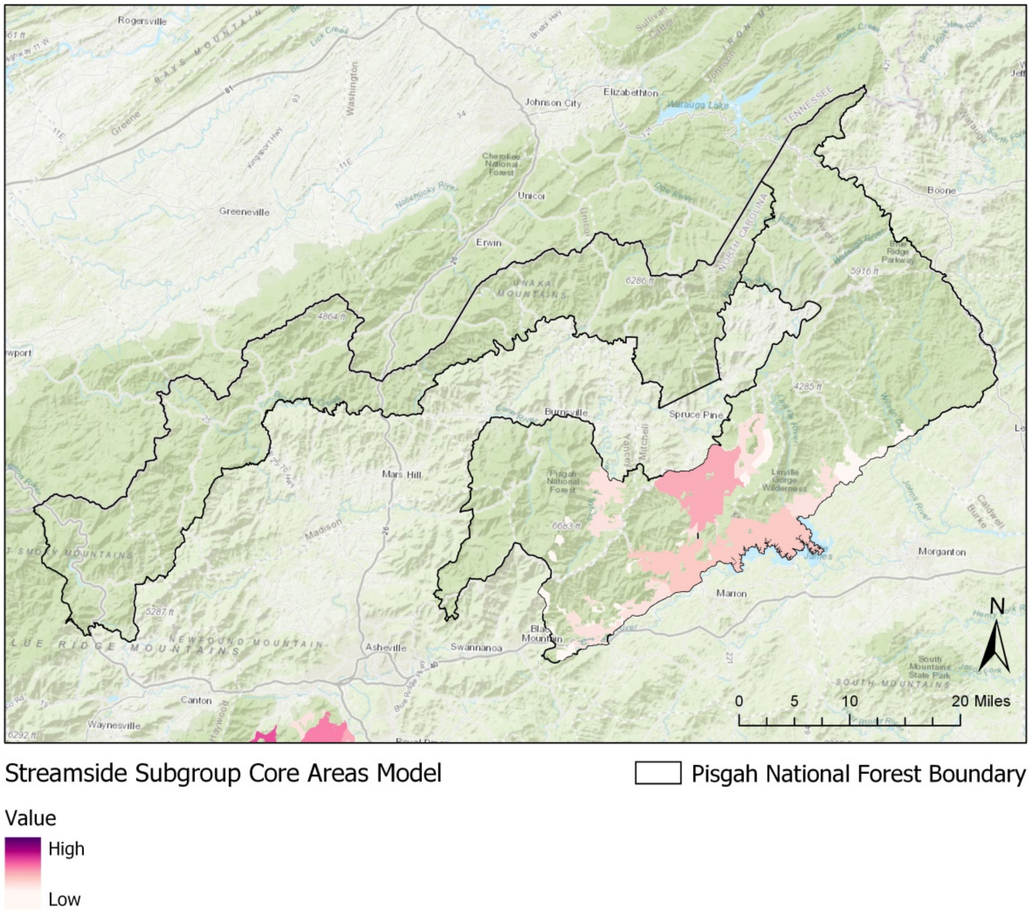


Figure 9. Map showing forest stands within the Pisgah National Forest containing core areas for the Streamside Subgroup as determined by the Core Area Model. Stands containing core areas with a higher priority value are dark purple while stands containing core areas with a lower priority value are light pink.

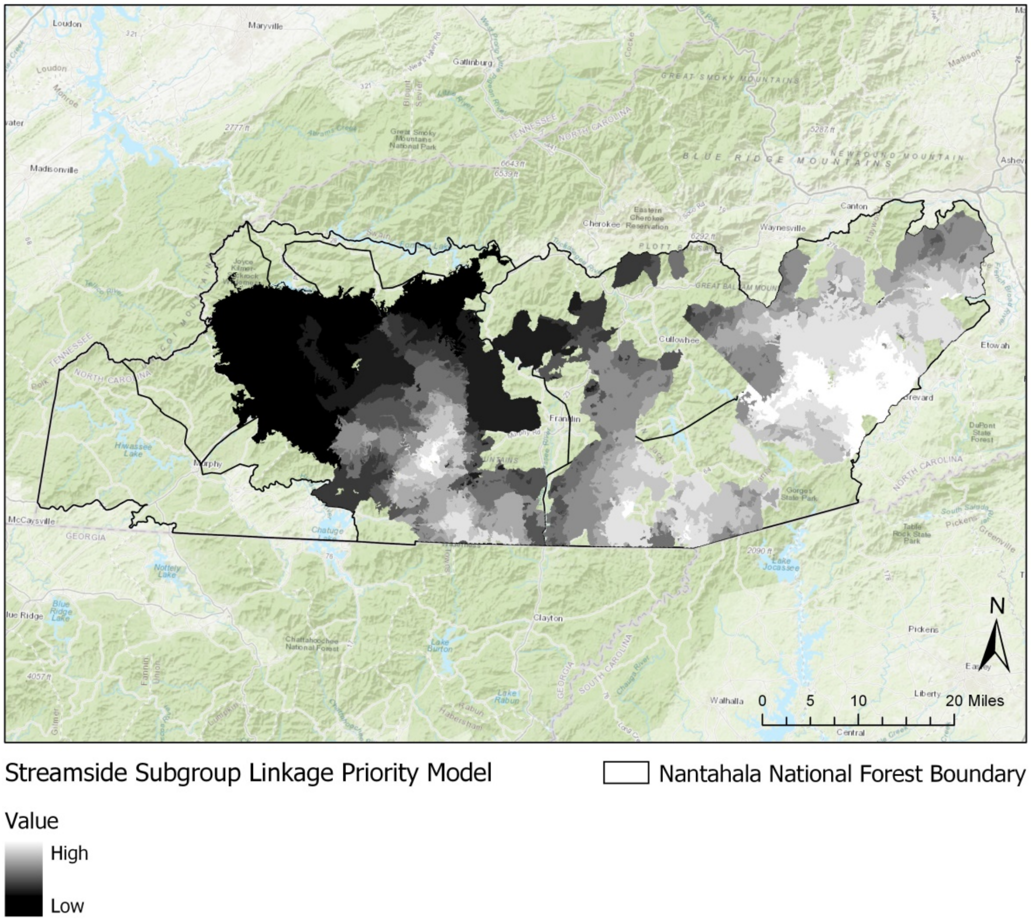


Figure 10. Map showing forest stands within the Nantahala National Forest containing linkages between cores for the Streamside Subgroup as determined by the Linkage Priority Model. Stands containing linkages with a higher priority value are white while stands containing linkages with a lower priority value are black.

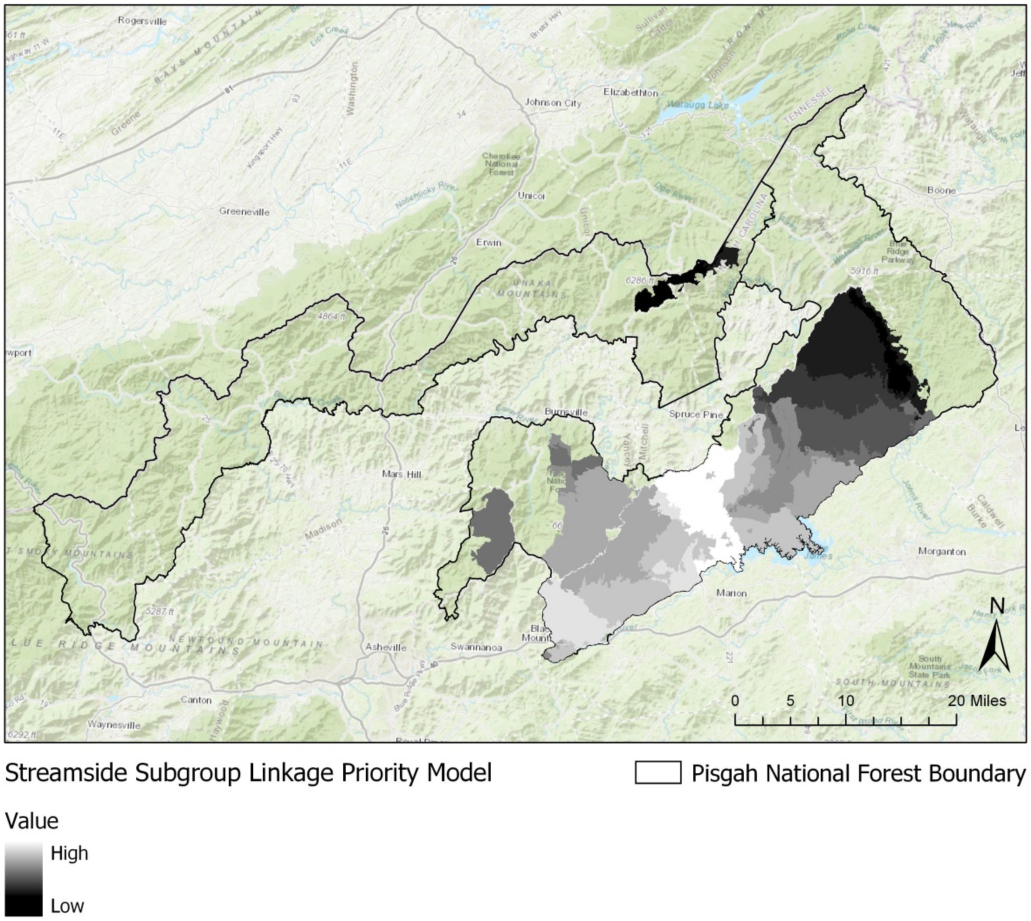


Figure 11. Map showing forest stands within the Pisgah National Forest containing linkages between cores for the Streamside Subgroup as determined by the Linkage Priority Model. Stands containing linkages with a higher priority value are white while stands containing linkages with a lower priority value are black.

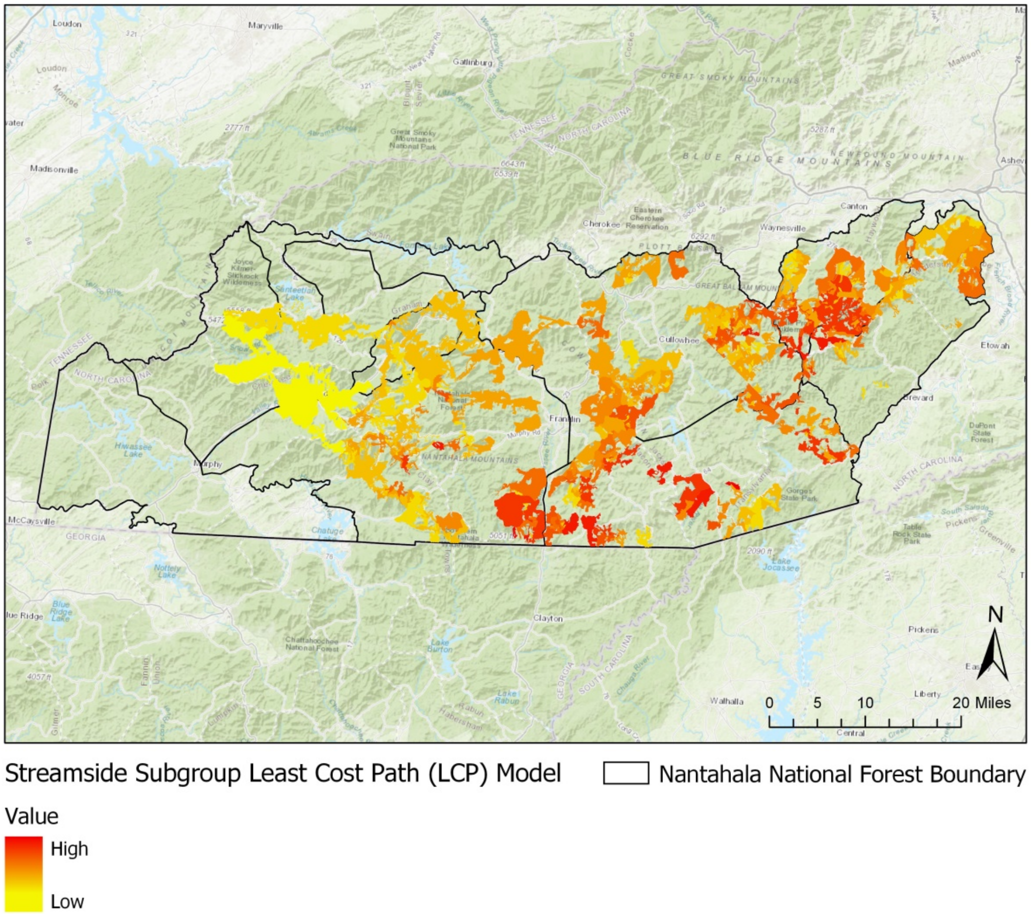


Figure 12. Map showing forest stands within the Nantahala National Forest containing least cost paths between cores for the Streamside Subgroup as determined by the Least Cost Path (LCP) Model. Stands containing paths with a higher priority value are red while stands containing paths with a lower priority value are yellow.

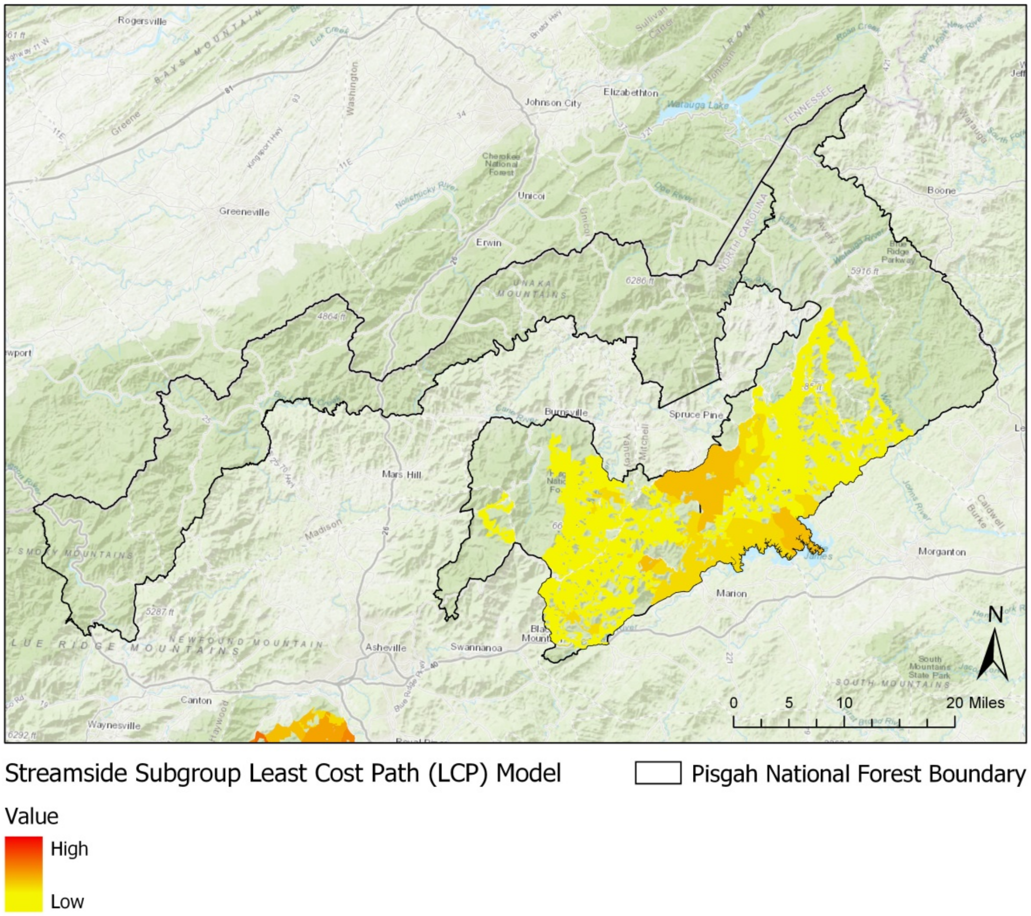


Figure 13. Map showing forest stands within the Pisgah National Forest containing least cost paths between cores for the Streamside Subgroup as determined by the Least Cost Path (LCP) Model. Stands containing paths with a higher priority value are red while stands containing paths with a lower priority value are yellow.

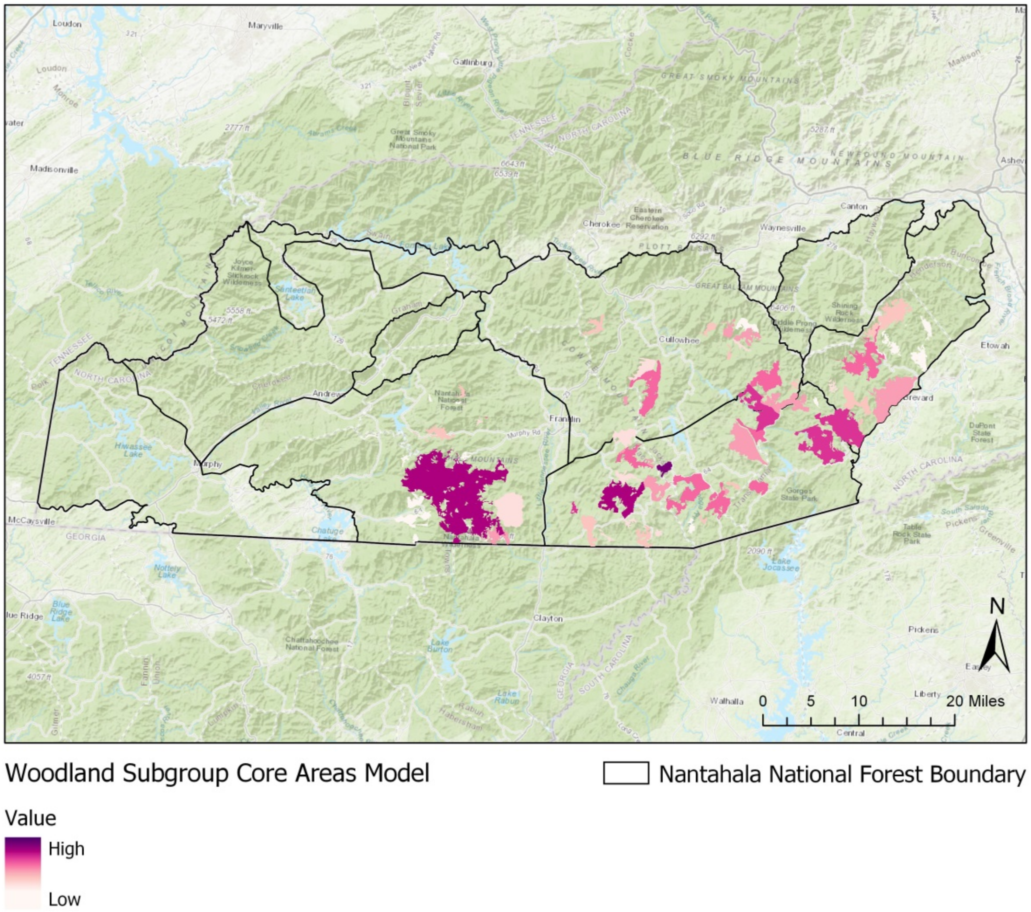
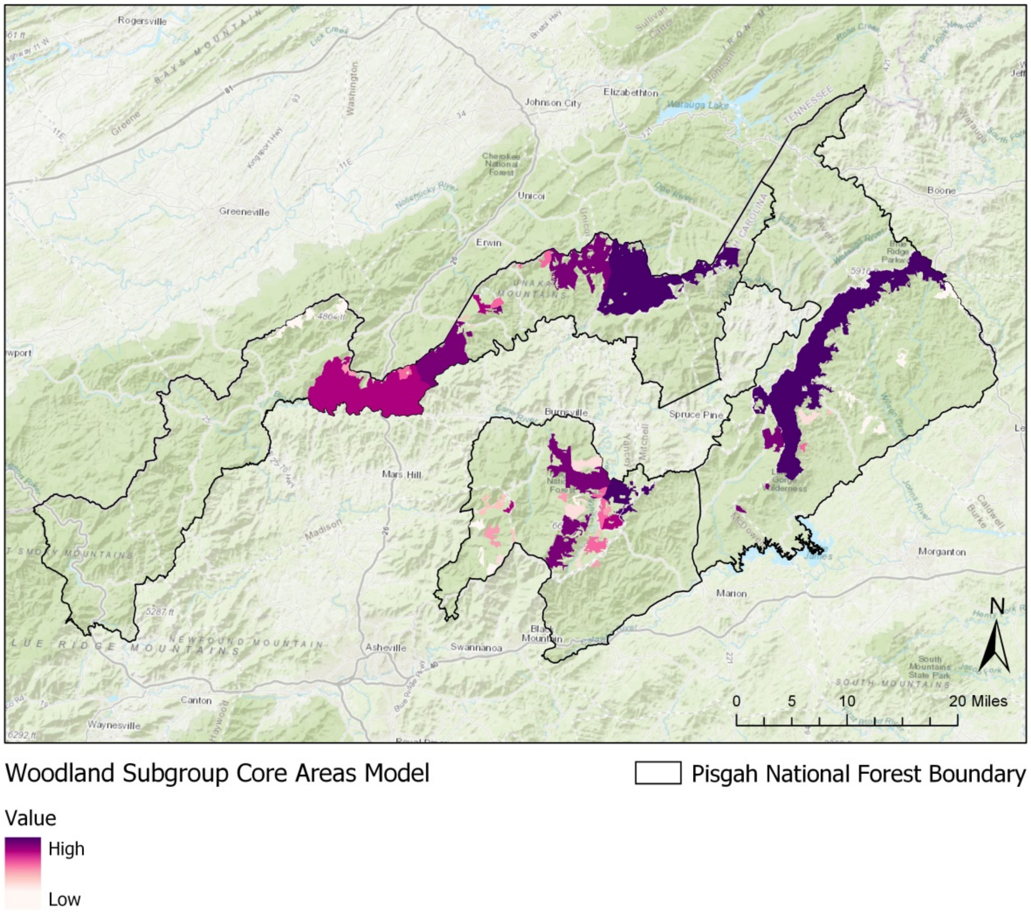


Figure 14. Map showing forest stands within the Nantahala National Forest containing core areas for the Woodland Subgroup as determined by the Core Area Model. Stands containing core areas with a higher priority value are dark purple while stands containing core areas with a lower priority value are light pink.



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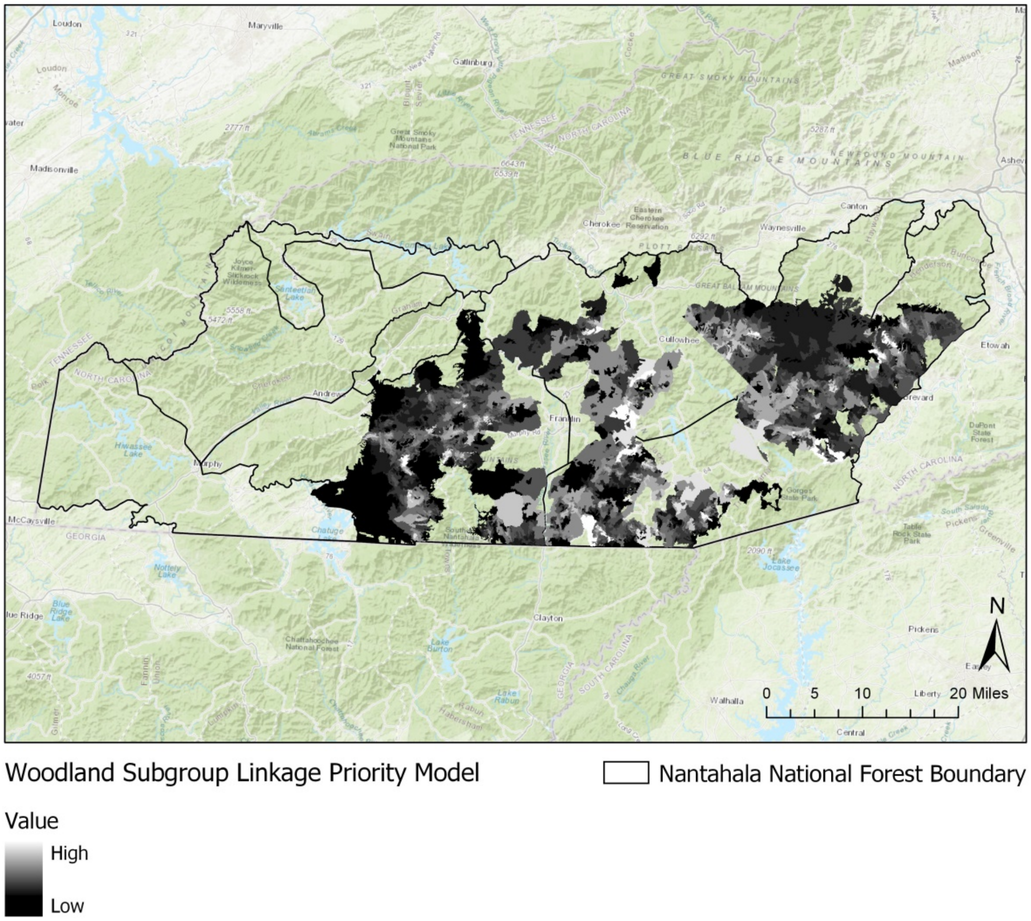

Figure 16. Map showing forest stands within the Nantahala National Forest containing linkages between cores for the Woodland Subgroup as determined by the Linkage Priority Model. Stands containing linkages with a higher priority value are white while stands containing linkages with a lower priority value are black.

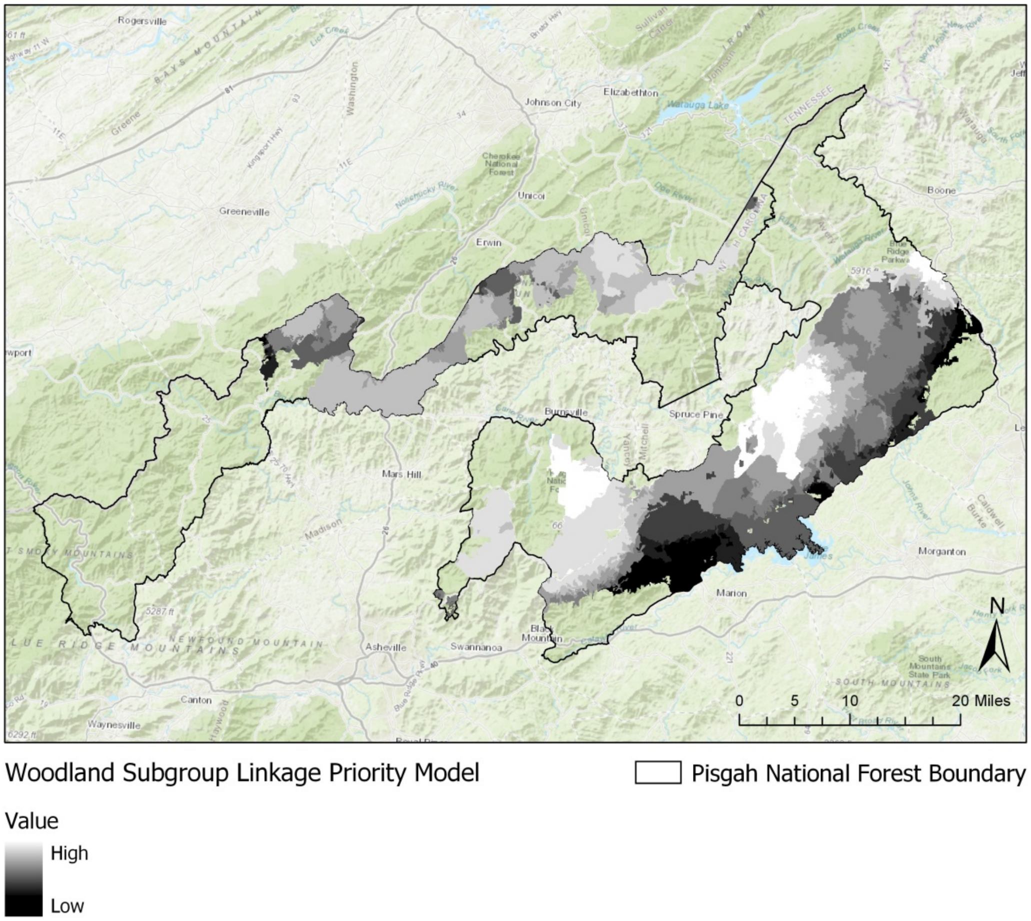

Figure 17. Map showing forest stands within the Pisgah National Forest containing linkages between cores for the Woodland Subgroup as determined by the Linkage Priority Model. Stands containing linkages with a higher priority value are white while stands containing linkages with a lower priority value are black.

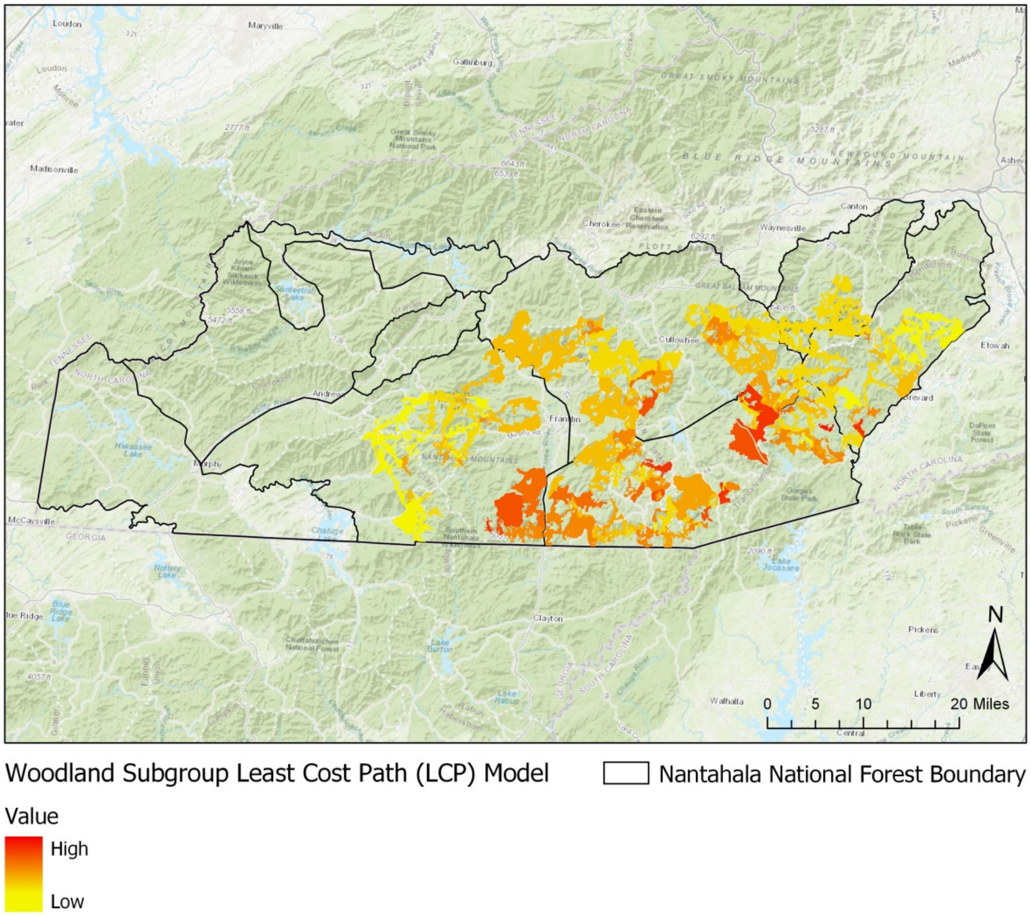

Figure 18. Map showing forest stands within the Nantahala National Forest containing least cost paths between cores for the Woodland Subgroup as determined by the Least Cost Path (LCP) Model. Stands containing paths with a higher priority value are red while stands containing paths with a lower priority value are yellow.

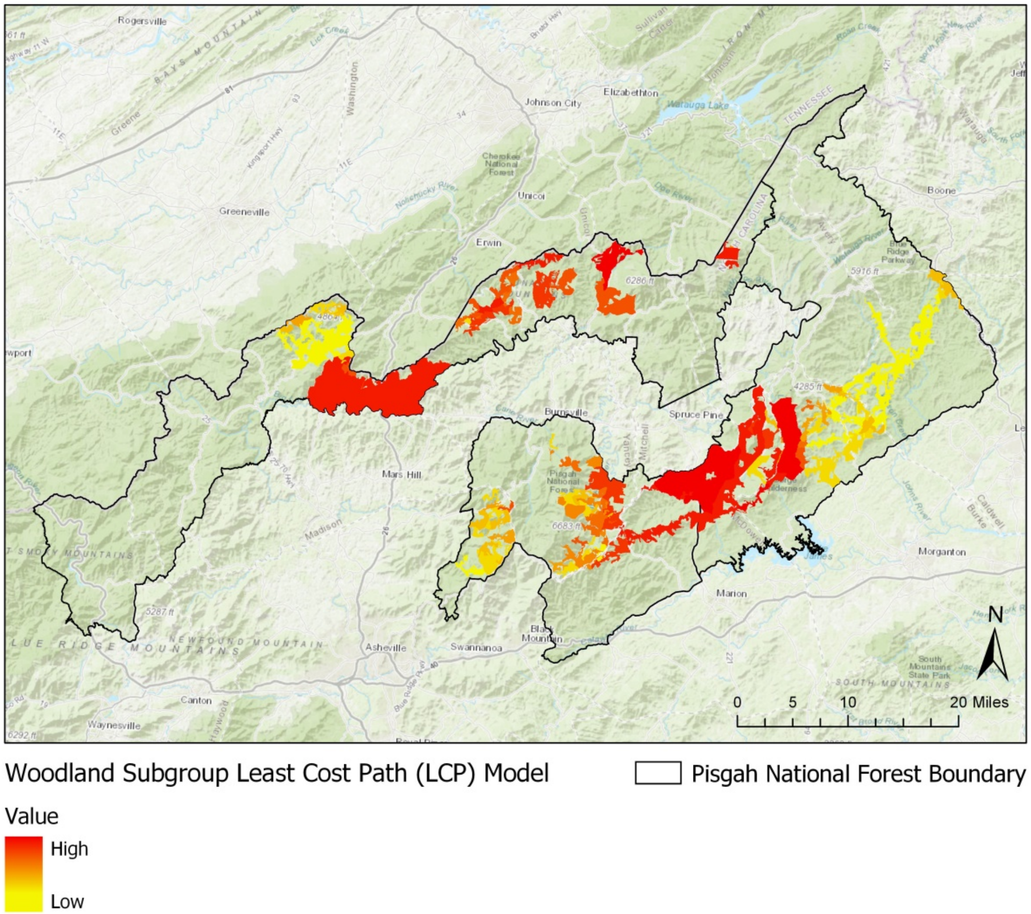

Figure 19. Map showing forest stands within the Pisgah National Forest containing least cost paths between cores for the Woodland Subgroup as determined by the Least Cost Path (LCP) Model. Stands containing paths with a higher priority value are red while stands containing paths with a lower priority value are yellow.

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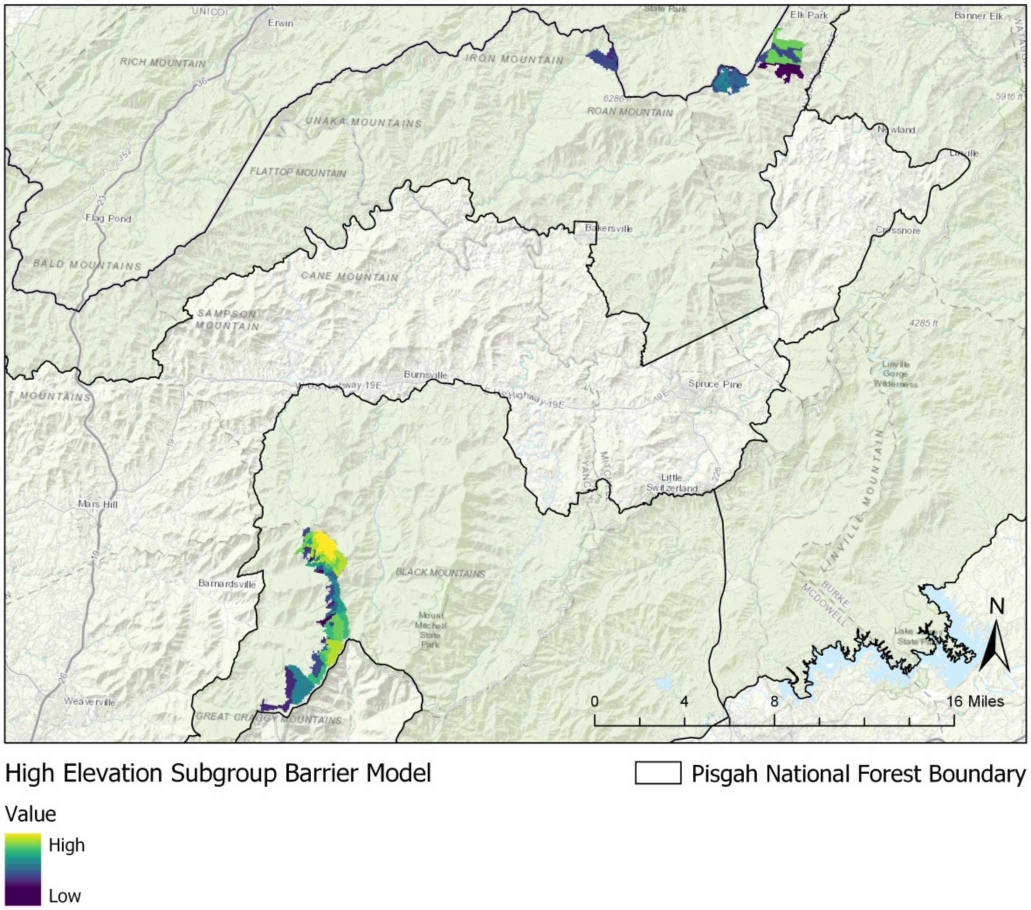
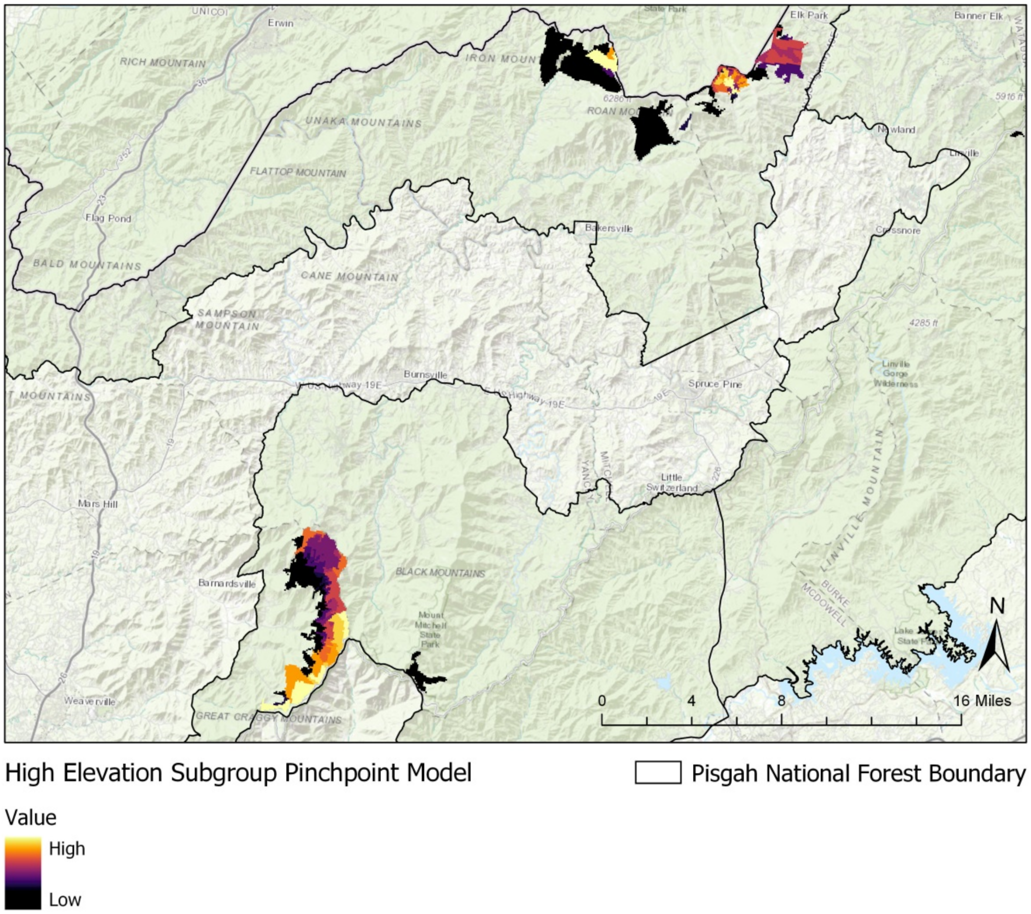
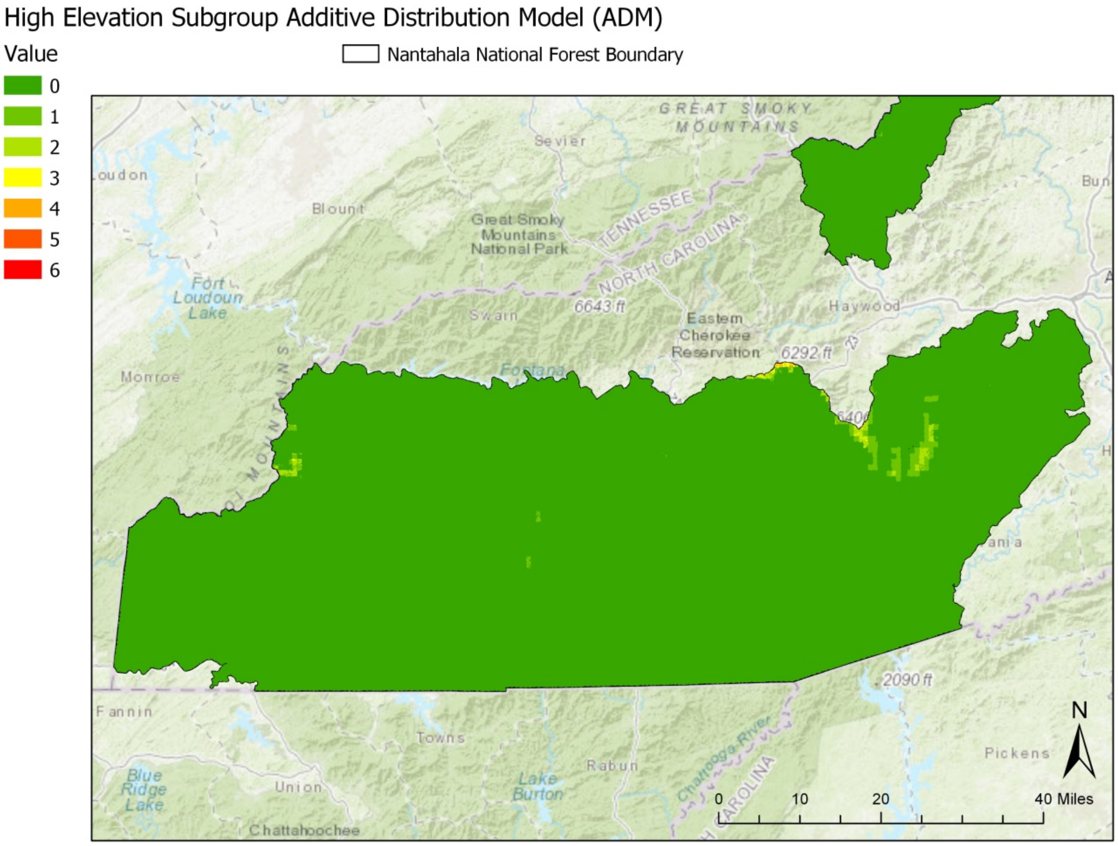
Appendix



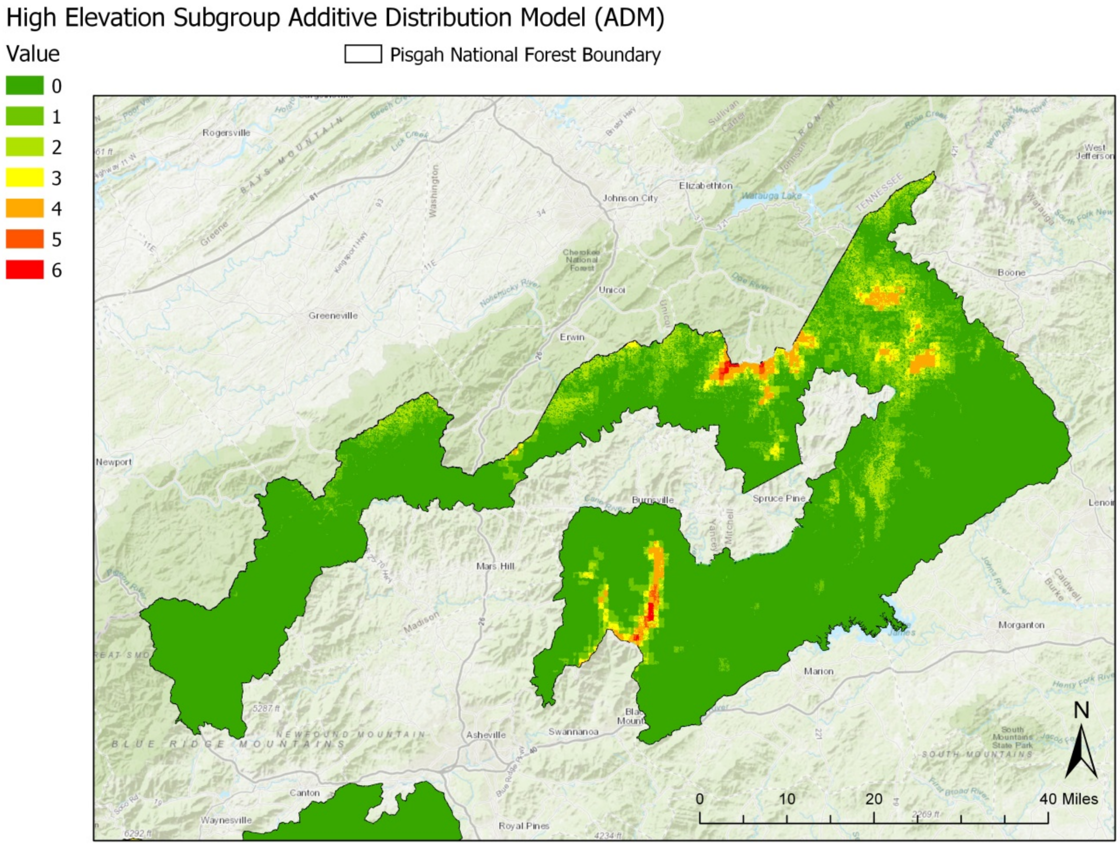
Supplementary Figure 1. Map showing forest stands within the Pisgah National Forest containing barrier centers for the High Elevation Subgroup as determined by the Barrier Model. Stands containing barrier centers with a higher value (corresponding to a higher reduction in least-cost distance per unit distance restored) are yellow while stands containing paths with a lower value (corresponding to a lower reduction in least-cost distance per unit distance restored) are dark blue.



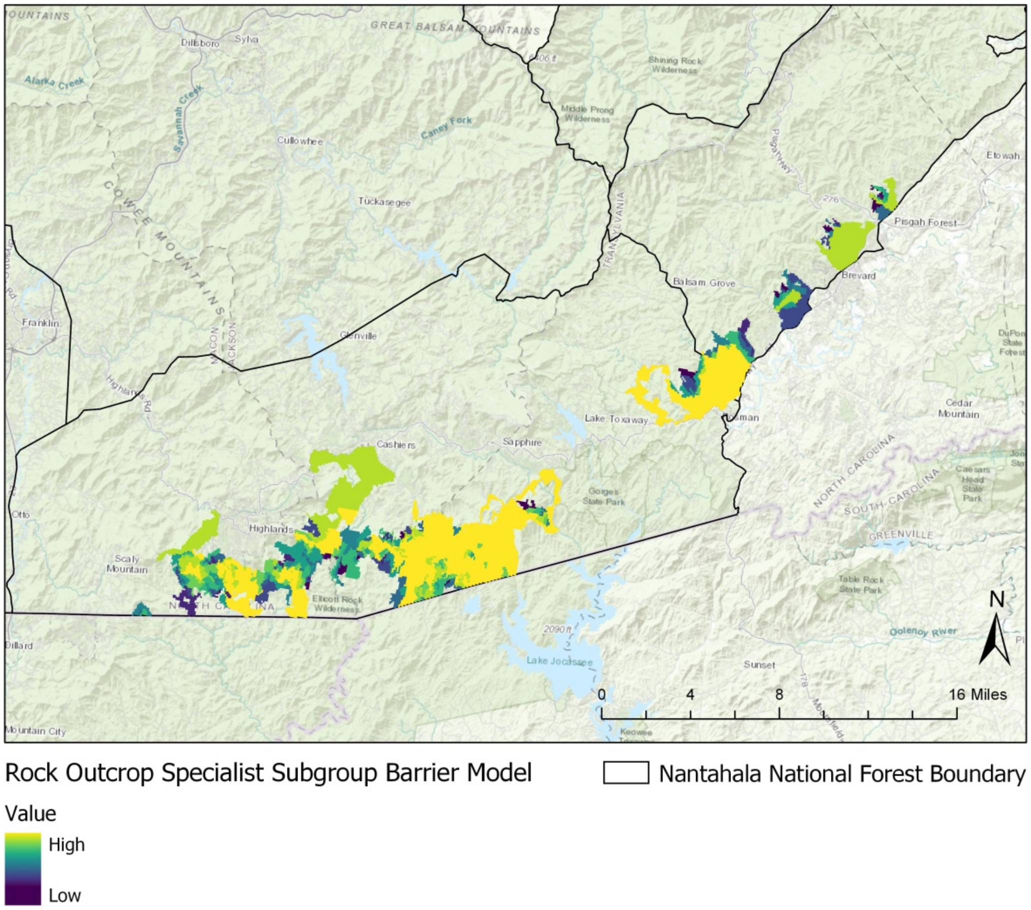
Supplementary Figure 2. Map showing forest stands within the Pisgah National Forest containing routes between cores for the High Elevation Subgroup. The value for these routes was determined using the Pinchpoint Model by running a current between cores and determining the relative resistance value of each route. Stands containing routes with a higher value (corresponding to a low resistance) are yellow while stands with a lower value (corresponding to a high resistance) are black.



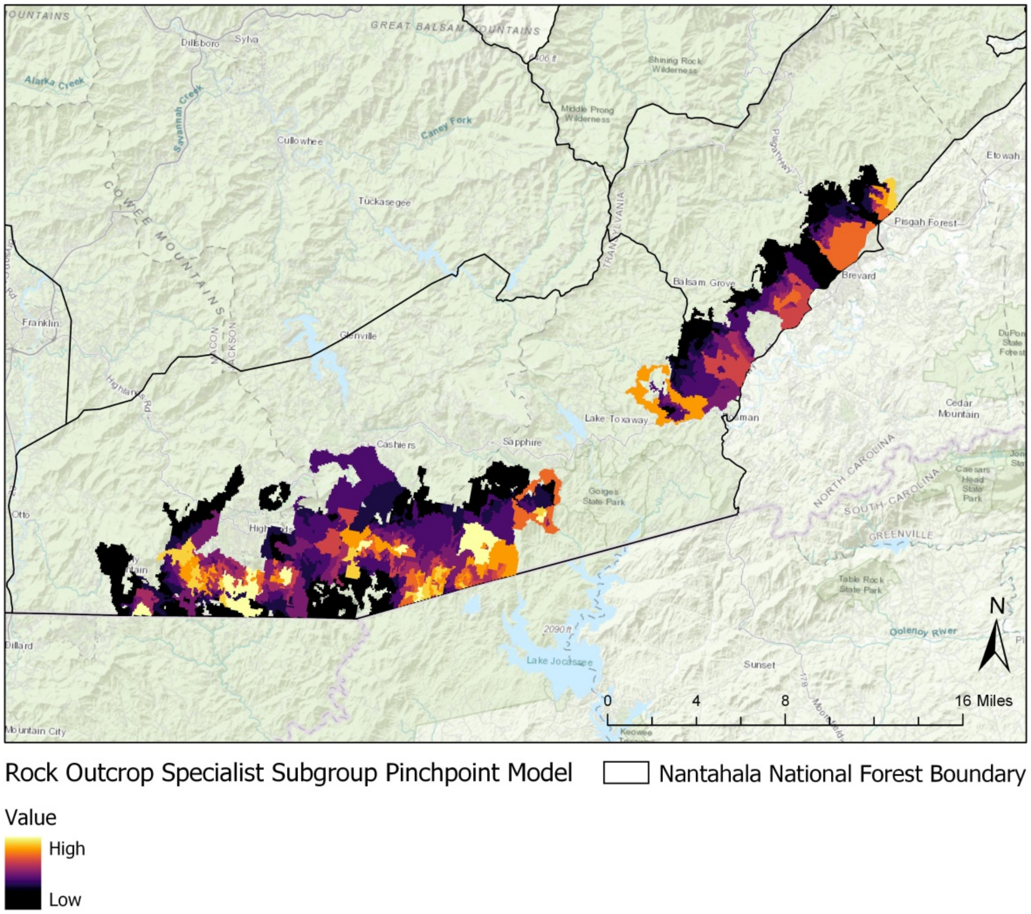
Supplementary Figure 3. Map showing the results of the Additive Distribution Model (ADM) for the High Elevation Subgroup in the Nantahala National Forest. Pixels with high values correspond to more suitable habitat and are red, while pixels with low values correspond to less suitable habitat and are green.



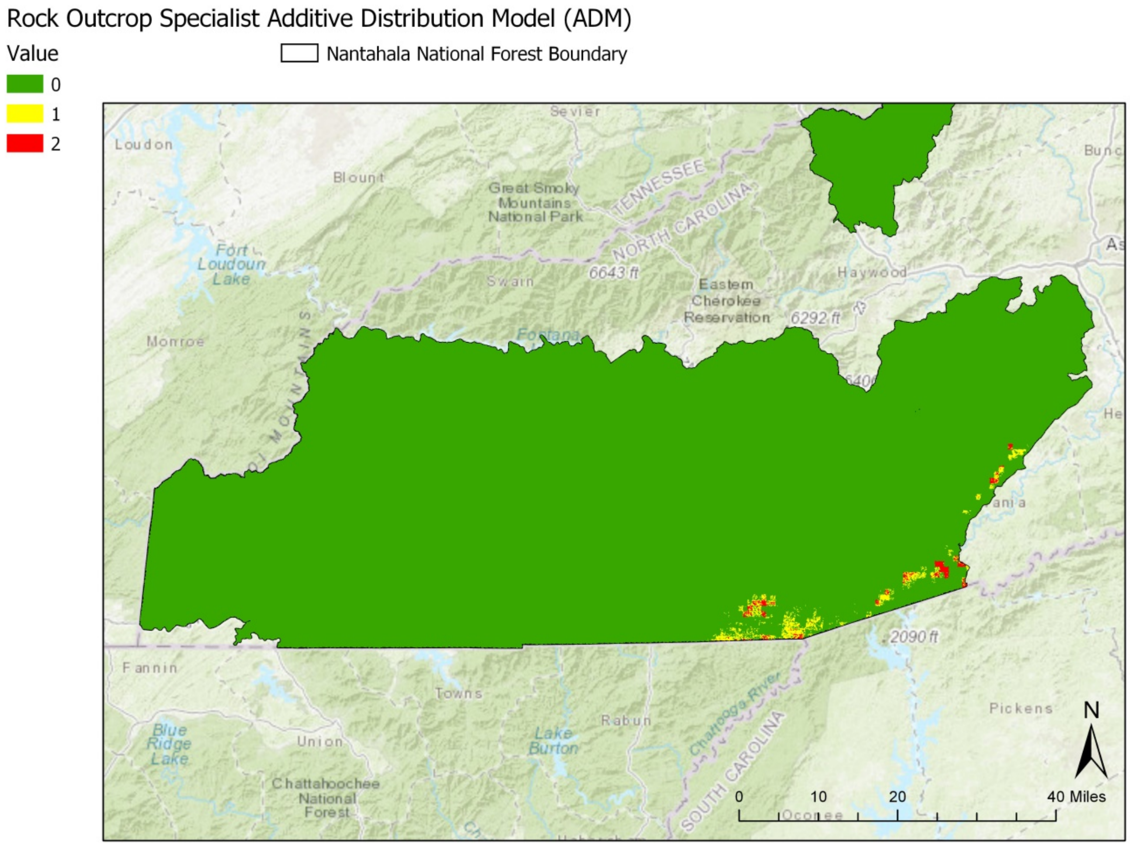
Supplementary Figure 4. Map showing the results of the Additive Distribution Model (ADM) for the High Elevation Subgroup in the Pisgah National Forest. Pixels with high values correspond to more suitable habitat and are red, while pixels with low values correspond to less suitable habitat and are green.



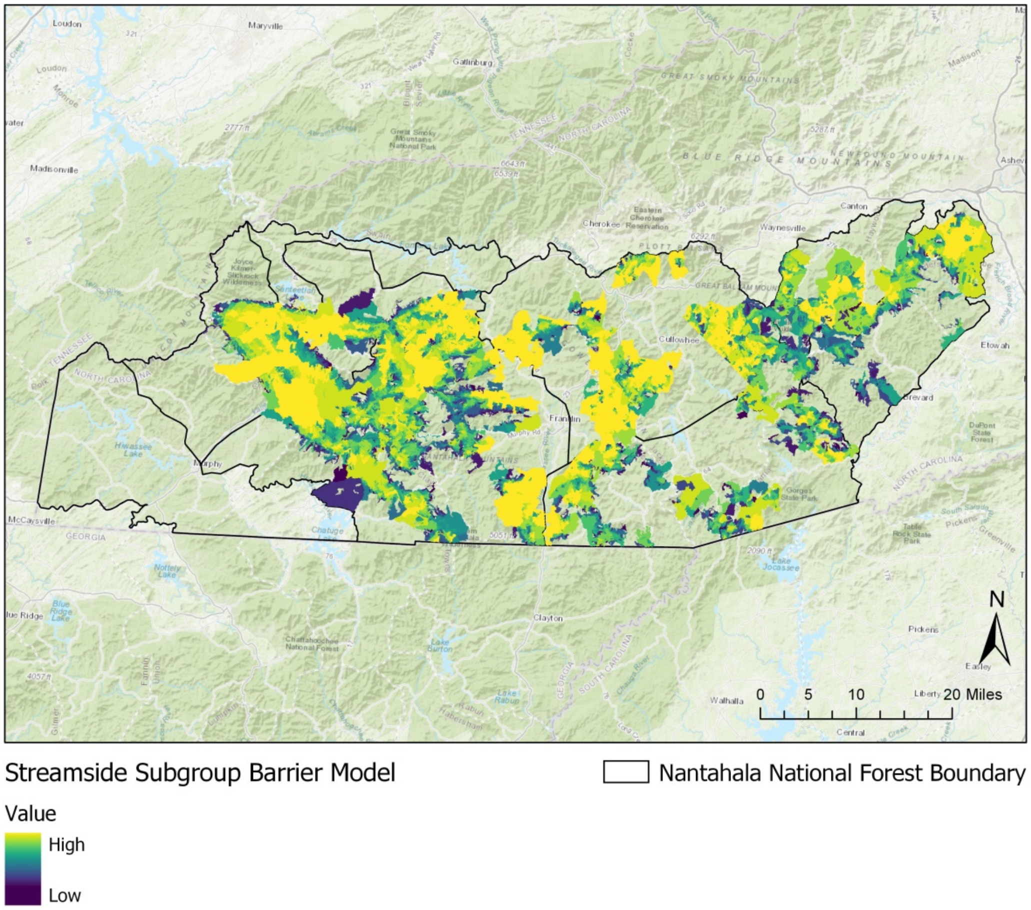
Supplementary Figure 5. Map showing forest stands within the Nantahala National Forest containing barrier centers for the Rock Outcrop Specialist as determined by the Barrier Model. Stands containing barrier centers with a higher value (corresponding to a higher reduction in least-cost distance per unit distance restored) are yellow while stands containing paths with a lower value (corresponding to a lower reduction in least-cost distance per unit distance restored) are dark blue.



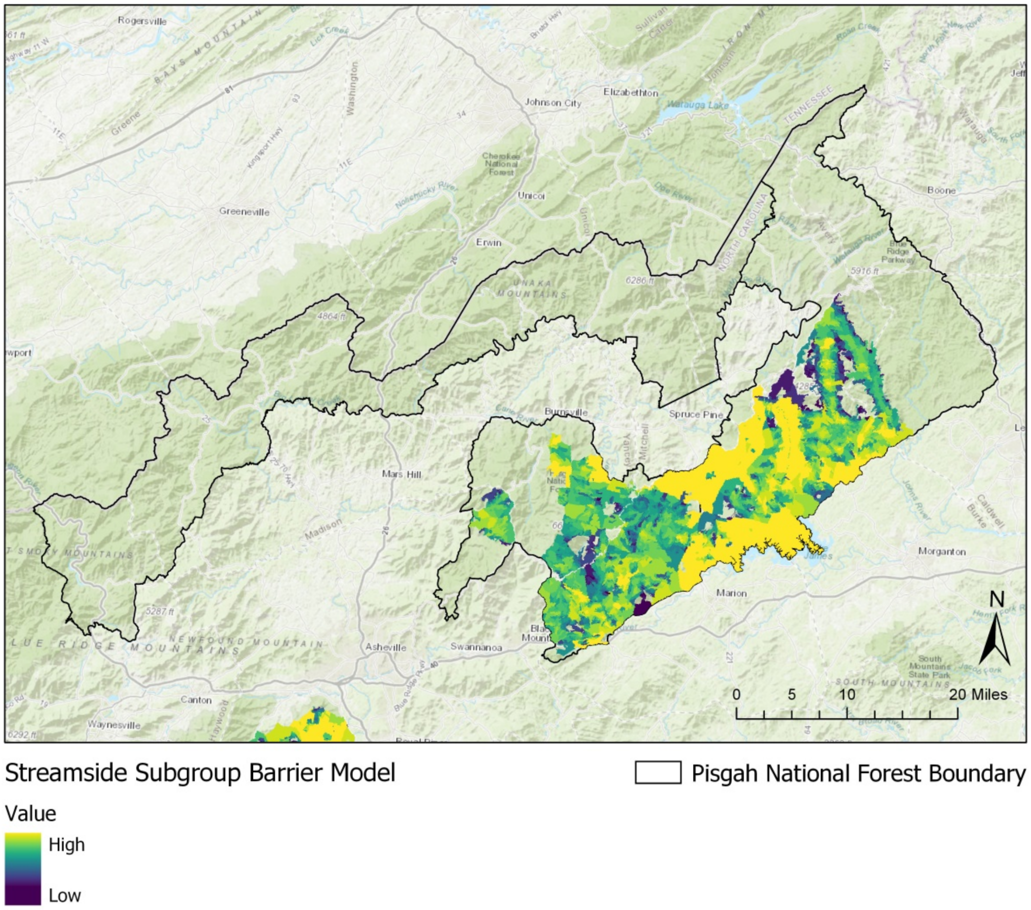
Supplementary Figure 6. Map showing forest stands within the Nantahala National Forest containing routes between cores for the Rock Outcrop Specialist Subgroup. The value for these routes was determined using the Pinchpoint Model by running a current between cores and determining the relative resistance value of each route. Stands containing routes with a higher value (corresponding to a low resistance) are yellow while stands with a lower value (corresponding to a high resistance) are black.



Supplementary Figure 7. Map showing the results of the Additive Distribution Model (ADM) for the Rock Outcrop Specialist Subgroup in the Nantahala National Forest. Pixels with high values correspond to more suitable habitat and are red, while pixels with low values correspond to less suitable habitat and are green.



Supplementary Figure 8. Map showing forest stands within the Nantahala National Forest containing barrier centers for the Streamside Subgroup as determined by the Barrier Model. Stands containing barrier centers with a higher value (corresponding to a higher reduction in least-cost distance per unit distance restored) are yellow while stands containing paths with a lower value (corresponding to a lower reduction in least-cost distance per unit distance restored) are dark blue.



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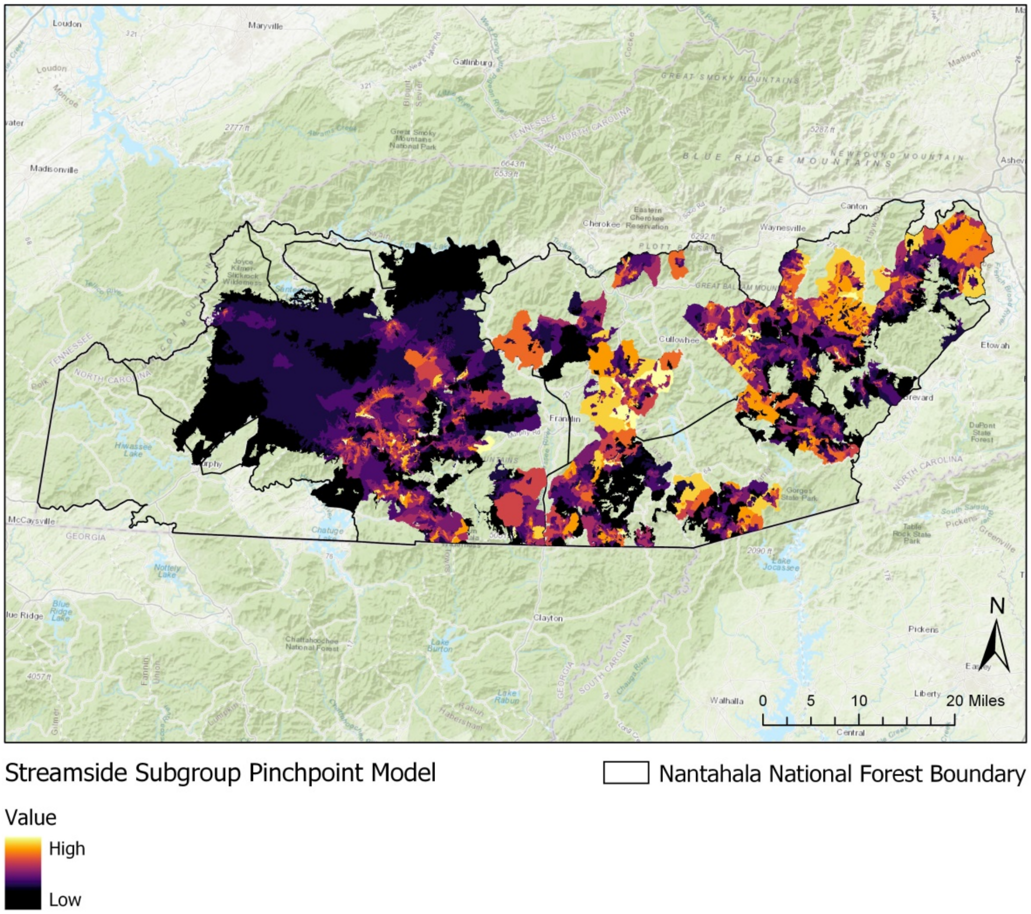

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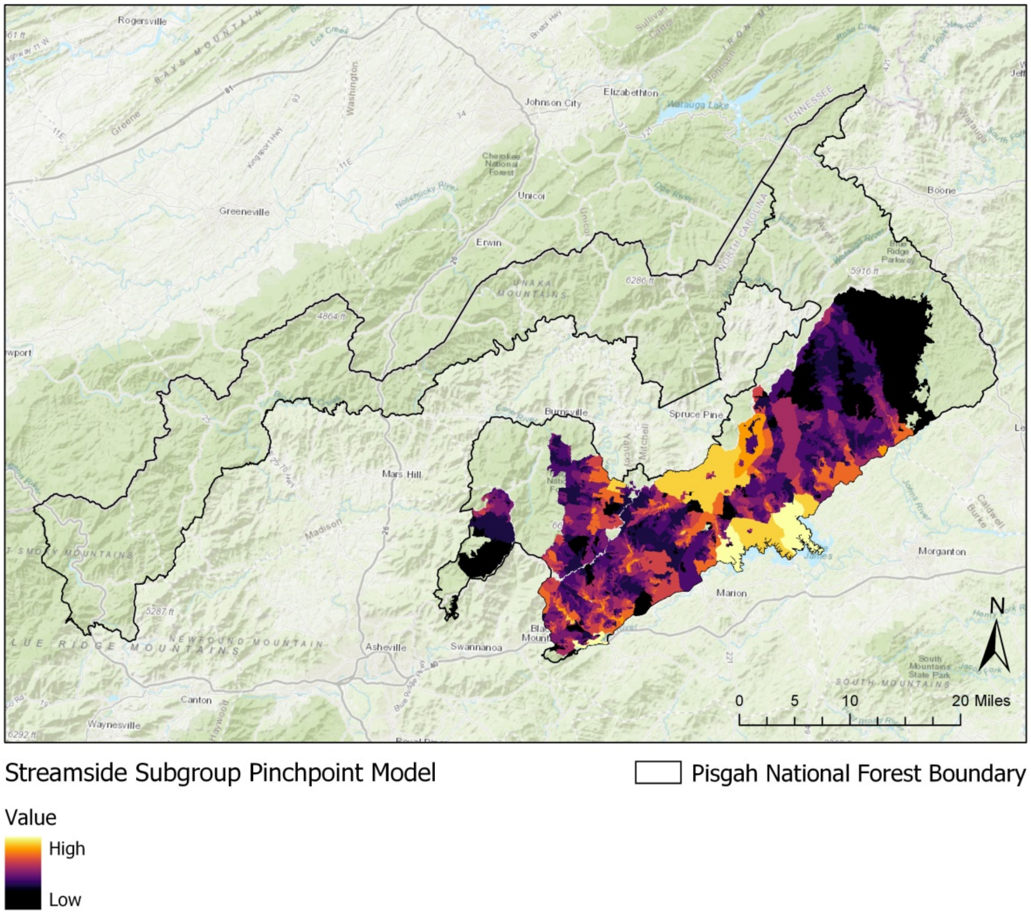

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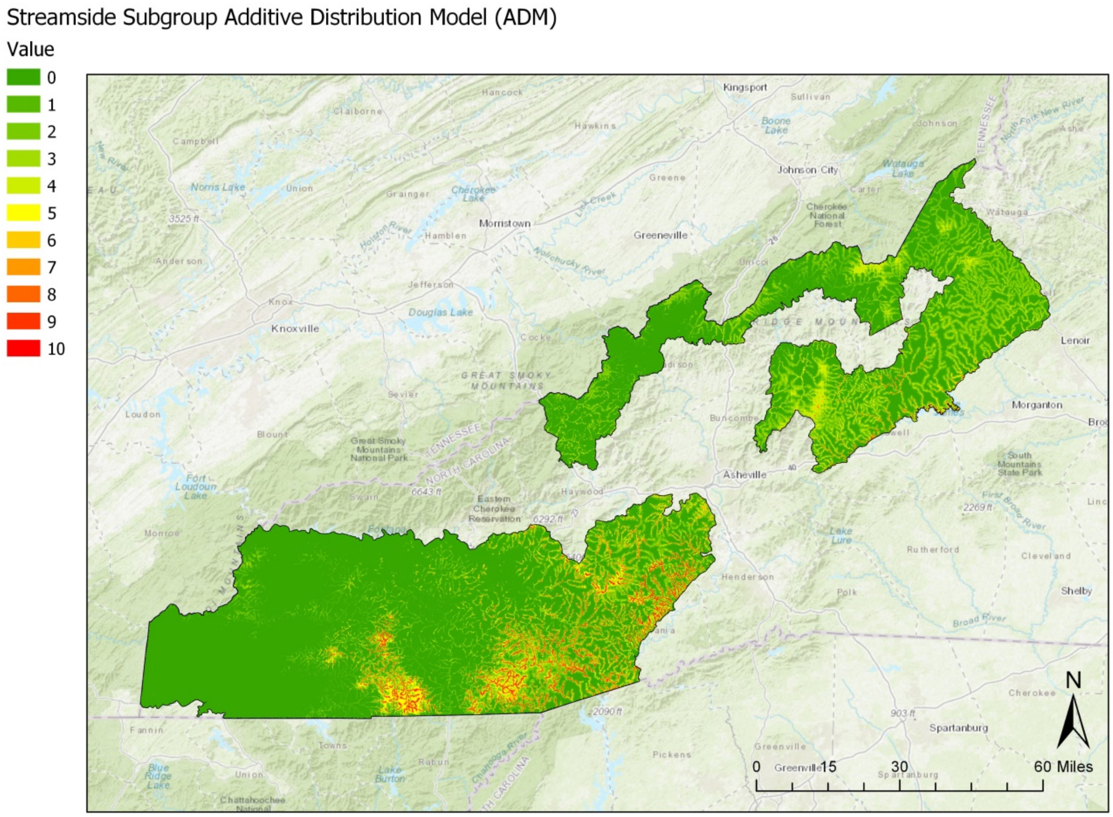

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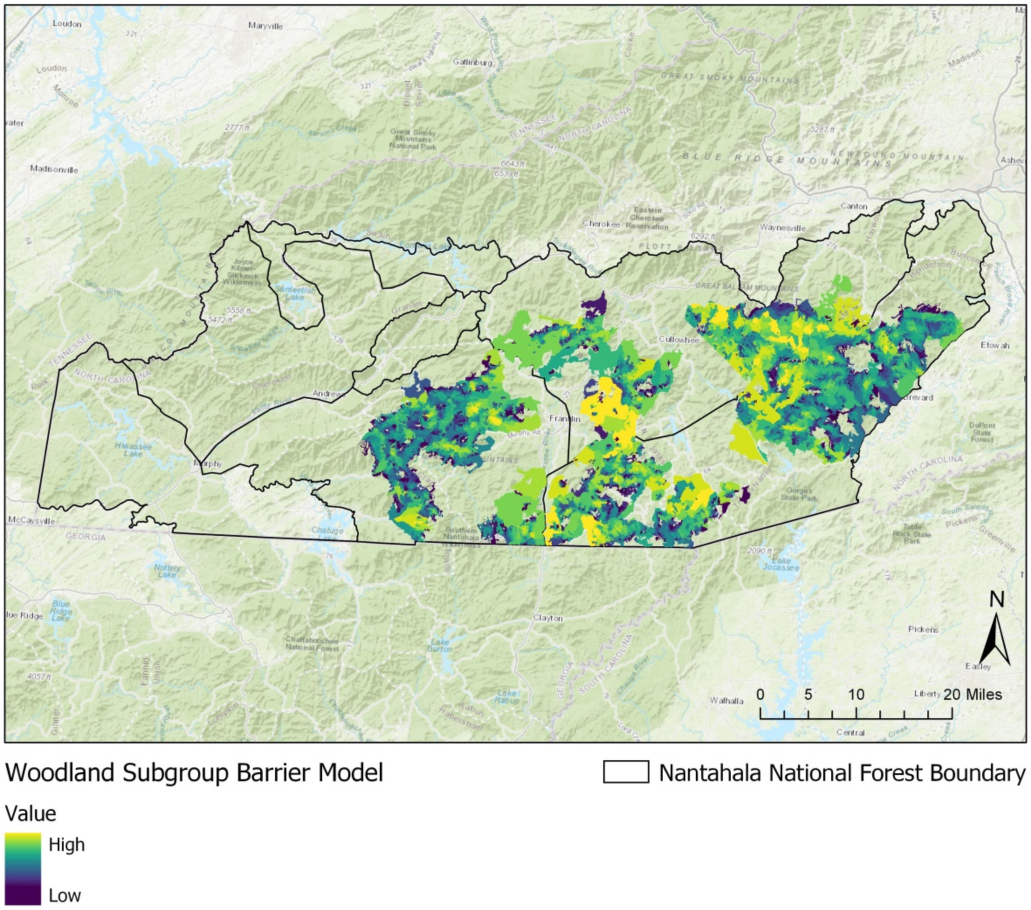

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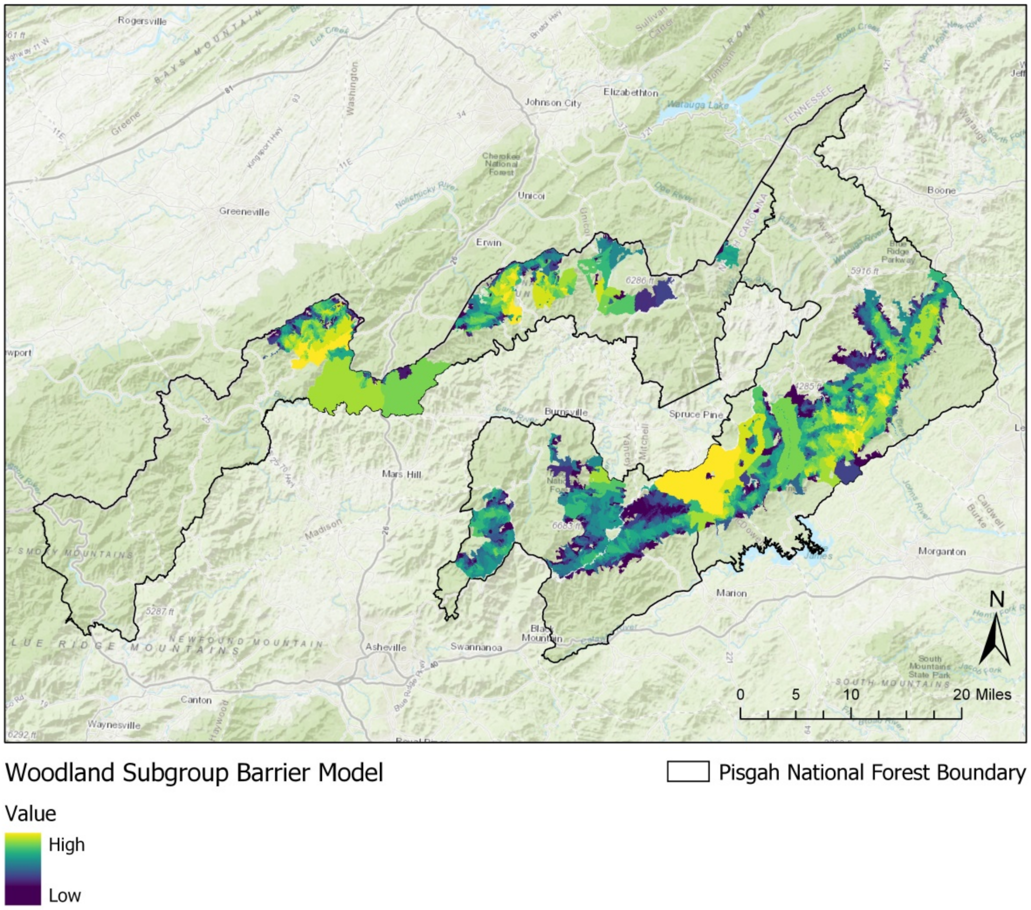

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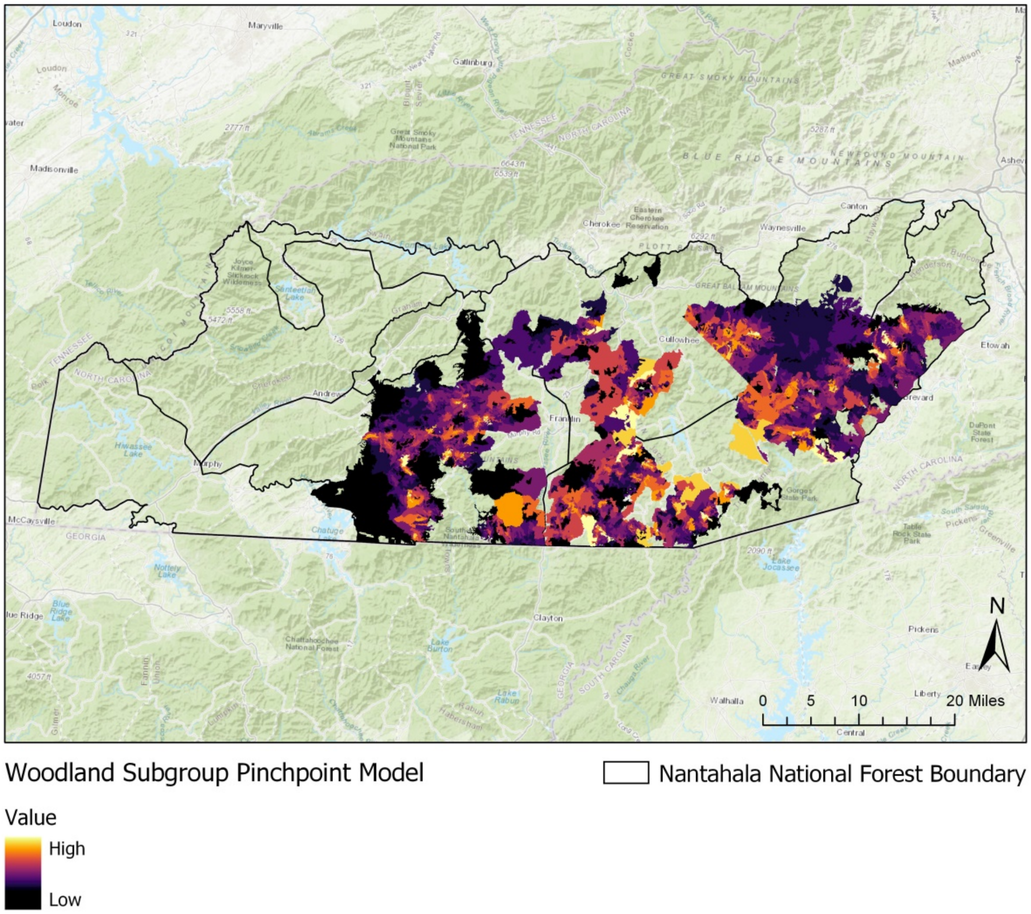

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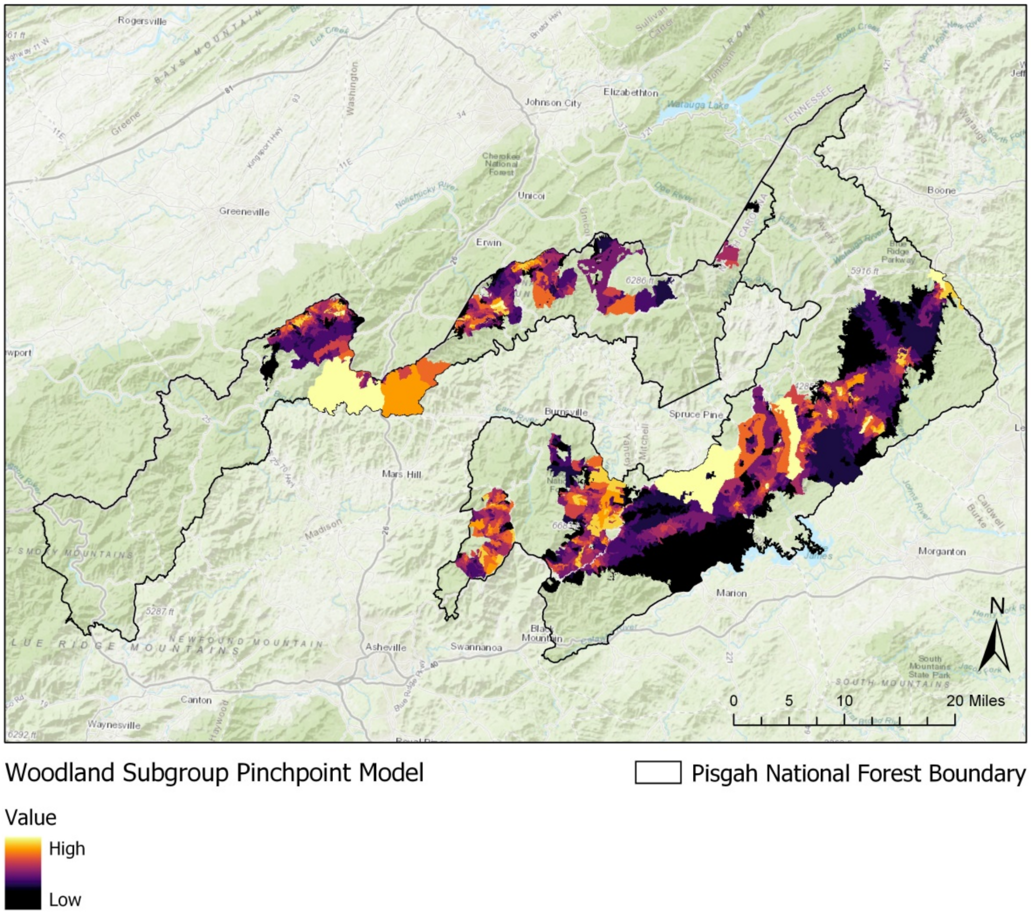

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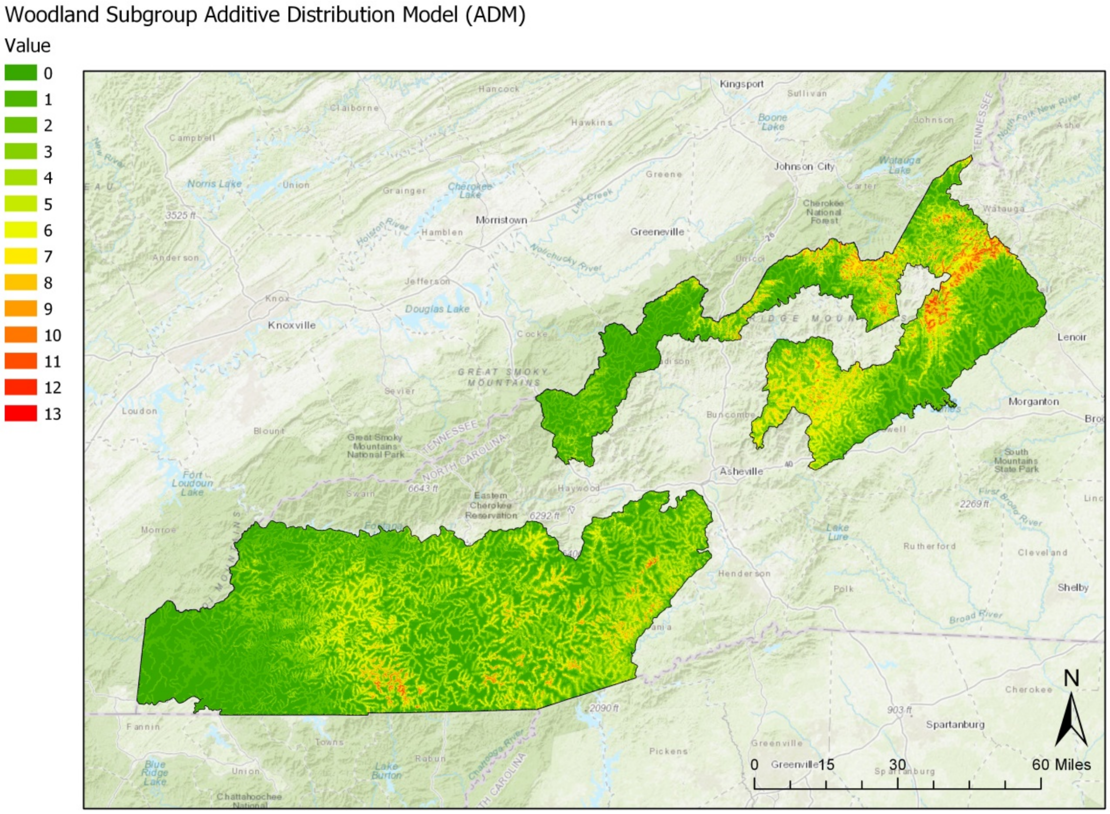

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Attachment 15

The Northern Flying Squirrel (*Glaucomys sabrinus*): A Conservation Challenge

Journal of Mammalogy, 88(4):897–907, 2007

THE NORTHERN FLYING SQUIRREL (*GLAUCOMYS SABRINUS*): A CONSERVATION CHALLENGE

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The northern flying squirrel (*Glaucomys sabrinus*) has an extensive range in North America, inhabiting boreal, coniferous, and mixed forests of the northern United States and Canada and the slopes of the mountains of the east and west. Most undisturbed northern populations are apparently thriving, but those in the southern mountains are considered disjunct relicts occupying declining remnants of suitable habitat. It is clear that range contraction in the past has been associated with climate and vegetation change in the Pleistocene and the large-scale timber harvests of the early 20th century and that today a significant threat comes from forest practices and development. However, the major problem in dealing with conservation of this species is understanding its complex ecological position in its regional communities and the subtle as well as obvious influences of human activities. Thus, to preserve this species over its extensive range one will have to consider its various roles as a biological opportunist, an important prey item, a disperser of mycorrhizae, a potential victim of biological warfare, and a small, secretive glider especially vulnerable to anthropogenic and possible climatic changes in the size, arrangement, and quality of its home forests.

Key words: conservation, *Glaucomys*, heterothermy, northern flying squirrel, *Strongyloides*, truffles

The ability to develop an effective conservation strategy for a vulnerable species presupposes that one knows enough about the animal's biology and the potential threats in its environment to create a meaningful protection plan. In the case of the northern flying squirrel (*Glaucomys sabrinus*), both the acquisition of adequate data and their interpretation have been a challenge. Although concern for this species over much of its range in North America has stimulated a great number of studies over the past 20 years after a long period of limited interest, the listing of some populations as endangered fueled an intense search for that ''magic'' factor or formula that might explain its biology, guarantee its survival, and eliminate its interference with the human exploitation of its home forests. We still have much to learn. As a participant in a symposium held at the annual meeting of the American Society of Mammalogists in June 2006, I was asked to address the broad problem of flying squirrel conservation. Although this topic may be approached in a number of ways, I have chosen to attempt to provide an overview—with pertinent background and examples—of 2 interacting components of this conservation issue: the particular or salient ecological factors potentially critical to species survival; and those human activities, past and present, contributing to the species' vulnerability. I am looking for common denominators—factors important to varying degrees over the wide range and diverse habitats occupied by this species as well as special, regional threats, and I wish to raise questions about current ideas and assumptions. I maintain that in the field of northern flying squirrel conservation there may be no simple solutions but instead, within some common denominator of basic biology, an array of problems and possible management strategies dictated by regional variation in squirrel ecology and in the kinds of human influences.

With some chagrin I have recently realized that I started my studies of flying squirrels as a graduate student 43 years ago. Thus, I have decided to approach the topic partially from a personal point of view, stressing my own experiences as well as findings documented in the literature and derived from discussions with other researchers. Although my studies have included many other vertebrates over the years, I have been repeatedly drawn back to flying squirrel investigations as interesting questions and concerns have arisen. Along with a few other workers, I have become a ''marked man,'' because, over the past 25 years, inquiries have poured in from federal and state agencies, conservancies, consulting firms, and various business concerns. Everyone wants definitive information on flying squirrels in order to preserve rare or endangered squirrel populations, to find a rationale to protect threats to parks and especially significant forests, or to provide justifications for logging, road building, or development in or near the species' habitat. I would argue that the predicament of the northern

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flying squirrel is often too complicated and subtle for the pat answers these people request. Thus, I hope I can be forgiven for using my own experiences in the southern Appalachians as a starting point for a broad but not a definitive discussion of the species, linking these findings to much of the other North American research.

BACKGROUND

The northern flying squirrel is not uniformly threatened over its wide range across the boreal forests of North America and the montane and mixed forests of the south-trending mountains of the east and west (Wells-Gosling and Heaney 1984). Except in areas under heavy settlement and large-scale clear-cutting, this species is holding its own rather well in much of the northern part of its range. Its vulnerability is most pronounced in the mountain areas at the southern margins of its range—the southern Appalachians, Sierra Nevada, and Rocky Mountains.

It is quite clear from historical studies of climate and vegetation that the species has experienced a number of range contractions in the past (Arbogast 1999, 2007; Arbogast et al. 2005; Weigl 1968). During times of glacial advance in the Pleistocene, boreal forests repeatedly extended as broad southern peninsulas along the eastern and western mountains and even down the Mississippi Valley (Davis 1976; Delcourt and Delcourt 1981, 1987). One can assume, based on a few fossil records, that the northern flying squirrel then occupied a much larger southern range. The retreat of the glaciers starting 18,000 years ago would have confined squirrels to narrower strips of land and isolated massifs along the Appalachians and western mountains, but much of its remaining habitat was probably quite adequate. Then, in the late 19th and early 20th century the catastrophic clear-cut logging of Appalachian forests took place. Huge areas were denuded and burned over a short period of time—a process repeated in the west somewhat later (Loeb et al. 2000). From what we can surmise from species' habitat requirements this was a critical time of range contraction, disjunction, and probably population extinction in the mountains. However, it is unlikely that the public or even the biologists of the time were at all aware of the plight of the flying squirrels. Many of the subspecies considered endangered or rare today were unknown. Hall (see Hall and Kelson 1959) described *Glaucomys sabrinus lucifugus* of Utah in 1934, Miller (1936) described *G. s. fuscus* of West Virginia in 1936, and Handley (1953) described *G. s. coloratus* of North Carolina and Tennessee in 1953. Although some populations from the west were described in the 1890s, many subspecies remained undiscovered until well into the 20th century (Hall and Kelson 1959; Howell 1918).

Starting in the early 1980s the northern flying squirrel became the object of intensive research, but much of this work concentrated on the more abundant and widely distributed northwestern forms, whereas the rare, relict, often inaccessible populations of the mountain ridges to the south received only limited attention in spite of the listing of some subspecies as endangered in 1985 (Weigl et al. 1999). Fortunately, recent studies have provided much more background information, and the pace of research has accelerated. However, we still have much to learn about the peculiarities of the ecology of this species and both the obvious and subtle effects of human activity. And that is why conservation of this species is such a challenge.

NORTHERN FLYING SQUIRREL ECOLOGY

General

In the simplest terms one can describe the northern flying squirrel as a small, nocturnal, nonhibernating, gliding tree squirrel that occupies boreal conifer and mixed forests and uses both tree cavities and dreys for nesting (Smith 2007; Wells-Gosling and Heaney 1984). Contrary to suggestions that this squirrel is a narrow, boreal specialist, the northern flying squirrel is best described as a behaviorally plastic opportunist, capable of adjusting its biology to wide range of conditions. For example, it is quite capable of occupying deciduous and lower-elevation woodlands of the east and west, not just the spruce, fir, and other conifer forests usually cited in the literature (B. S. Arbogast, pers. comm.; Weigl et al. 2002; Weigl and Osgood 1974). Faced with cold temperatures, turbulent weather, and short periods of food limitation, the squirrel can become heterothermic, dropping its body temperature several degrees without becoming torpid (Bowen 1992). This enables it to wait out short intervals of bad weather and make the most of its body energy reserves. Unlike most squirrels, it does not depend on seeds and nuts, even when these are available (Brink 1965; Brink and Dean 1966; Hall 1991; Mitchell 2001; Thysell et al. 1997), but, although occasionally using mast, generally subsists on fungi, lichens, buds, berries, staminate cones, and animal material, none of which it appears to store. Even its reproductive biology is rather flexible. Although the squirrel commonly produces a litter in early spring, in some areas energy availability and condition of females lead either to reproductive failure or delay, with litters being observed late into the summer and even into October or December (Raphael 1984; Weigl et al. 1999; Witt 1991, 1992). Thus, compared to the smaller southern flying squirrel (*G. volans*) and most other North American tree squirrels, *G. sabrinus* possesses some unusual ecological characteristics, in keeping with the diversity of environmental conditions it must survive.

What salient features of the ecology of the northern flying squirrel need to be considered in developing conservation measures? Our knowledge of the species is still quite fragmentary, because relatively few long-term studies have been conducted (Carey et al. 1999; Cotton and Parker 2000a, 2000b; Fryxell et al. 1998; Lehmkuhl et al. 2006; Ransome and Sullivan 2002; Smith and Person 2007; Weigl et al. 1999). Most studies have been of short duration, confined to warmer months, or limited to surveys. Long-term, year-round investigations are rare. In addition, once some populations were listed as endangered in 1985 in the Appalachians and others were deemed vulnerable because of habitat modifications in the west, researchers avidly attempted to acquire and interpret new data in a quest for unitary and perhaps overly simple strategies to preserve these squirrels. Because the extensive literature on the genetics, biogeography, and ecology have been largely reviewed by Arbogast (2007) and Smith (2007) in this issue, I will concentrate on aspects of the squirrel's biology that appear essential to conservation of the species and then raise questions about the current state of our knowledge and interpretations. Some of my comments will be based on the literature, some on personal experiences.

Habitat

In reviewing the voluminous literature on the habitats utilized by northern flying squirrels, one cannot help but be impressed by certain common features as well as some regional variations that perhaps reinforce this perceived ''common denominator'' (Waters and Zabel 1995; see Smith 2007). Northern flying squirrels generally occupy boreal or north temperate conifer, mixed conifer–hardwood, and northern hardwood forests, as found in the northern United States and Canada, at various elevations of mountain regions, and in some narrow valleys subject to cold air drainage. These habitats support old-growth forest, communities with old-growth elements, or younger woodlands usually contiguous with such forest. Such areas are usually cool and moist, have cold winters, and possess a well-developed canopy, substantial ground cover, quantities of wet, dead, and downed wood, and often organic substrates. These conditions favor an abundance of snags, cavities, witches brooms, trees festooned with lichens and moss, and a diverse array of buds, berries, seeds, and fungi. In drier sites in the west, squirrels appear to select riparian areas where these cooler and wetter conditions prevail, and where there is easy access to drinking water (Meyer et al. 2005, 2007). In fact, Carey (1989, 1995) observed differences in population densities in Washington and Oregon that might be associated with moisture conditions in various forest types. Although one can point out variations in this ''typical'' habitat description, it is clear that the northern flying squirrel is versatile enough to prosper in a wide range of forest types as long as the above conditions occur in enough favorable patches and enough habitat is left undisturbed.

Although *G. sabrinus* may be a habitat opportunist and readily uses a diversity of potentially suitable forests, habitat is a major conservation problem, exacerbated by various controversial approaches to forest management. The ongoing harvest of old-growth forest, its replacement with plantations or regenerating stands, and the increasing fragmentation of much of the remaining habitat has alarmed some biologists concerned about this and other rare animal species (see Smith 2007). When rare species are declared endangered, as in the case of the northern flying squirrel, then economic forces exert tremendous pressure on researchers to develop definitive management plans that will protect the rare organisms, but also allow a return to timber harvest and development. Such is the case in Alaska (Smith and Person 2007) where the size, quality, and connectedness of planned reserves is an issue, in the Pacific Northwest where the debate over the importance of old growth versus successional forests to rare species has raged for years (Carey 1989, 1995; Lehmkuhl et al. 2006; Waters and Zabel 1995; Witt 1992; but see Ransome and Sullivan 1997, 2002, 2004; Rosenberg and Anthony 1992), and in the Sierra Nevada where thinning, fire, and harvesting may limit the size and quality of squirrel habitat (Meyer et al. 2005; Meyer and North 2005). Another example comes from the Appalachians where the currently endangered subspecies *G. s. fuscus* of West Virginia is a candidate for delisting. In the Appalachians northern flying squirrels are commonly found in older forests of spruce (*Picea rubens*), fir (*Abies fraseri*), beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), and yellow birch (*Betula alleghaniensis*), especially in the ecotones between conifers and hardwoods. However, throughout the east from Nova Scotia, Canada (Lavers 2004), to southern North Carolina (Weigl et al. 2002) the species is known to occupy hardwood habitats without spruce and fir. An array of studies have documented the squirrel's habitat diversity (Ford et al. 2004; Menzel et al. 2006; Payne et al. 1989; Stihler et al. 1987; United States Department of the Interior, Fish and Wildlife Service 2006; Urban 1988) pointing out the importance of hardwood and mixed forest habitats. *G. sabrinus* of West Virginia is more abundant and its populations more continuous than in most parts of the east. Many of the squirrels are caught in forests in which spruce is present, and this tree species supports one of the fungal genera (*Elaphomyces*) eaten by the squirrel (Loeb et al. 2000). Therefore, the United States Fish and Wildlife Service has decided that if forests containing spruce are protected in the national forests, the flying squirrel's preservation is insured, and it can be delisted, not to the ''threatened'' level but taken off the critical list entirely. The problems with this approach are many. First, it is not clear if there is any direct causality between the presence of flying squirrels and spruce. Both animal and plant may be responding independently to the same boreal conditions. Squirrels may nest in spruces occasionally and use them as one of many food sources, but there is no proof of any obligate relationship. Second, in more than 40 years of trapping and nestbox checking in various Appalachian habitats, I almost never captured animals in extensive, pure conifer stands, although telemetry revealed that they sporadically used them. Third, such a course of action fails to sufficiently protect the northern hardwood areas often used by *G. sabrinus*. Finally, the quality and connectedness of the proposed spruce-containing reserves, now and in the future, need careful study, especially in a region where timber harvest is an important part of the local economy. My main point is that economic pressures may at times influence how ecological information is interpreted resulting in overly simplistic solutions to a conservation and political issue.

Foods

One of the especially significant aspects of northern flying squirrel ecology and conservation is the direct link between the squirrel, its diet, and the perpetuation of its forest habitats. Years ago, McKeever (1960) noted high levels of fungi in the guts of California animals, and in 1965 I discovered that North Carolina squirrels were consuming large quantities of fungi and the staminate cones of fir (Weigl 1968). Subsequently, research in the Pacific Northwest documented the dependence of northern flying squirrels on the fruiting bodies of hypogeous, mycorrhizal fungi (truffles—Carey et al. 2002; Fogel and Trappe 1978; Lehmkuhl et al. 2004; Maser and Maser 1998; Maser et al. 1978, 1985, 1986; Meyer and North 2005; North et al. 1997; Pyare and Longland 2001b). The hyphae of these underground fungi form associations with tree roots, greatly increasing their surface area for the absorption of water and minerals at a small energy cost to the tree. Many tree species grow poorly or not at all without mycorrhizae. But spore dispersal to new seedlings and older trees is a problem for an underground fungus. Based on our study of the northern flying squirrel and another truffle eater, the fox squirrel (*Sciurus niger*—Weigl et al. 1989), and the work of Zabel and Waters (1997) and Pyare and Longland (2001a), the following scenario has taken shape. The truffle produces a fruiting body that gives off a chemical signal on ripening; this causes a squirrel to avidly excavate and devour the fungus (Secrest 1990). However, although the squirrel obtains energy and certain minerals (e.g., sodium and phosphorous) from these truffles, it is unable to digest the fungal spores, which are then dropped over the landscape for days or weeks afterward (Gamroth 1988). The resulting inoculation of young trees and spread of the fungus may thus have a marked impact on the perpetuation of the forest habitat on which the squirrel depends. Although *G. sabrinus* is not the only mycophagist in its home forest, it is one of the most mobile and spends much time on the ground during foraging (Bird and McCleneghan 2005; Loeb et al. 2000; Mitchell 2001; Zabel and Waters 1997). In any case, because of these food habits and their positive effect on the trees of its habitats, conservation of this species assumes a greater dimension and significance. In fact, many of the habitat models for *G. sabrinus* are now implicitly based on recognition of this squirrel, tree, and fungus symbiosis (Ford et al. 2004; Menzel et al. 2006; Odom et al. 2001; see Smith 2007).

Given the above account of the use of hypogeous fungi, it is important to link these and other foods to certain environmental factors. Truffles are the fruiting bodies of mycorrhizal fungi and appear to be most abundant in association with larger and older living trees, especially in moist, organic soils. The time course of fungal inoculation, growth, and maturation of sporocarps may vary in different forests, but old-growth conditions may be optimal. Epigeous fungi and lichens, which also are important foods, depend on abundance of dead wood and extensive tree surface areas, respectively, and, once again, cool, wet conditions. Although lichens and animal material such as insects and carrion may help support squirrels in the winter when most other foods are unavailable, some researchers also have found evidence for winter truffle use in habitats with frozen ground. Hackett and Pagels (2003) and Smith (2007) have data on the use of underground nests, but no one has reported underground foraging in winter. The other plant materials making up the squirrels diet—staminate cones, berries, beechnuts, and some seeds—are reflective of a preference for boreal habitats and old-growth conditions but also are indicative of an opportunistic species that is not limited to truffles and that might utilize additional foods.

Demographic Considerations

In spite of the spectacular increase in northern flying squirrel studies, we have surprisingly little information on the species' life history and population biology. Most studies have been dedicated to particular questions such as home range, relative density, foods, and habitat associations. Longer-term studies (e.g., Carey et al. 1999; Fryxell et al. 1998; Smith et al. 2004, 2005; Smith and Nichols 2003; Weigl et al. 1999) have begun to fill in some gaps in our knowledge, but we know very little about most population parameters and long-term temporal and spatial trends.

Smith and Person 2007 have recently reviewed much of the demography of the species and raised questions about the distribution and stability of populations. The picture of *G. sabrinus* that is developing is of a relatively long-lived (4–7 years) species with a low reproductive rate for a small mammal. In the western part of the range of *G. sabrinus*, flying squirrels appear to be more abundant than in the east and more continuous in their distribution within the old-growth forests that they commonly occupy. However, most workers report lower densities in managed or successional stands. In the east, populations often occur in distinct patches, often kilometers away from other groups in spite of what seems to be suitable intervening habitat (Weigl et al. 1999, 2002). Also in the east, population size appears to be highly variable. In some years, squirrels will be abundant in an area; in other years the populations are low or nonexistent. Have the animals died out or moved? No answer is available, but population fluctuations have been noted by other researchers (Fryxell et al. 1998). In spite of the meager data from recaptures, it is clear that at least some of the squirrels missing in intervening sampling sessions show up again months or years later (Weigl et al. 1999).

Examination of telemetry data from throughout North America suggests that home-range size is associated with habitat quality and food resources (Smith 2007). Home ranges from 2 to 60 ha have been reported. Our own work and that of others have revealed that squirrels have relatively small core home ranges (3–15 ha) that vary somewhat with sex and season, but that many individuals will display bouts of extensive linear travel, in some cases more than a kilometer, that involve both outward movement and return (Menzel et al. 2006; Weigl et al. 1999). There is some evidence that this long-distance travel is associated with a search for foods and possibly mates (Weigl et al. 1999). Such forays may affect home-range estimates if data are taken at wide time intervals. The important question here relates to the use of space by the species. If populations in a locality can fluctuate widely in numbers, have a distinctly patchy distribution in fairly uniform forest, and consist of individuals that can cover spectacular distances, it is possible that northern flying squirrels may use and thus require much larger expanses of suitable habitat than is commonly acknowledged if they are going to survive in many parts of their range. Both habitat size and connectedness assume great significance under these conditions.

Smith and Person 2007 have recently provided an intriguing example of space use that may partially relate to the preceding discussion. Working in Alaska in undisturbed habitat, they investigated populations in prime old-growth forest and adjacent groups in a wet, mixed muskeg and forest landscape. Examination of the demographic data suggested that there was a dynamic source–sink situation governing these populations. The muskeg areas were not maintaining viable squirrel populations in a steady state, but were the beneficiary of constant migration of animals from the better forest habitats. To what extent high mobility, source–sink conditions, and metapopulation distributions of squirrels are a common phenomenon is unknown, but this may be worth investigating in areas with old-growth forest adjacent to human-modified habitats. The squirrel populations reported from cutover and regenerating areas may be more variable because they are not self-perpetuating. Certainly the status of populations in West Virginia, the Sierra Nevada, and parts of the Pacific Northwest should be evaluated with this possibility in mind.

Other Species of Animals

The fate of northern flying squirrels may be closely linked to the presence of other animal species—predators, competitors, and parasites—that are in turn often of particular concern to wildlife biologists and conservationists.

Predators.—Smith (2007), Carey et al. (1992), and Weigl et al. (1999) have described some of the potential predators of the flying squirrel, but 2 in particular may be of interest in different parts of the range. Over the past 20 years it has become clear that the northern spotted owl (*Strix occidentalis*), an endangered and much celebrated species of western forests, is especially dependent on the northern flying squirrel as a prey item (Carey et al. 1992). The owl seems to thrive in extensive old-growth forests or in habitats with old-growth elements where the squirrels are most abundant (Carey 1995; Carey et al. 1999). The size and condition of the habitat ideal for supporting both the flying squirrel and the owl have been the focus of ferocious debate (Carey et al. 1992; Ransome and Sullivan 2002; Rosenberg and Anthony 1992). Old-growth forests in the west are becoming smaller in size and increasingly fragmented, but often are viewed as the economic salvation for a timber industry that is worried about an endangered species restricting the exploitation of remaining tracts. For the squirrel the issue of habitat quality, size, and connectedness is of great importance and has been the focus of several studies. Conservation of squirrel and owl thus seems inextricably linked, but doubtless shall remain a source of intense political and economic controversy.

In the eastern United States another rare animal is periodically associated with the issue of protection of *G. sabrinus*. Every few years, wildlife biologists consider the reintroduction of the fisher (*Martes pennanti*) to the southern Appalachians; this species was known to exist in the region in the recent past. In most areas fishers can probably coexist with northern flying squirrels without problems. But in small habitat islands of the southern Appalachians with few squirrels and limited alternate prey items, a predator such as the fisher might kill off these relict populations. Although there have been no introductions of fishers in areas with isolated flying squirrel populations, this idea resurfaces frequently (R. Powell, pers. comm.) and will require the careful attention of wildlife agencies in the region.

Competitors.—Smith et al. (2004, 2007) have suggested that the biology of *G. sabrinus* in the Pacific Northwest may be different from that in Alaska and the east because of the abundance of other small mammals in western forests. This diversity of sympatric rodents might then produce a greater degree of den-site and food specialization in response to direct and diffuse completion. In reality, we have little information on resource competition between northern flying squirrels and other mammals. Although red squirrels (*Tamiasciurus hudsonicus*) and Douglas squirrels (*T. douglasii*) are often mentioned as possible competitors, there is not much evidence of any severe interaction. Flying squirrels may pilfer food from red squirrel middens and the 2 species may both use cavities for nesting sites and fungi for food, but the very different overall diets of these squirrels and their nocturnal–diurnal activity separation may minimize interactions, especially in good habitat. In many years of trapping both species, I was always surprised to find that the best years for capturing northern flying squirrels also were the best for red squirrels.

The southern flying squirrel (*G. volans*) often has been considered a major competitor (Weigl 1968, 1978). Both species are nocturnal gliders that use tree cavities for dens and both may consume fungi, insects, and plant parts. Although experimental studies suggested that *G. volans* was the more active and aggressive in interactions, especially around nests (Weigl 1978), habitat preferences, diets, and climatic tolerances of the 2 species (Bowen 1992; Bowman et al. 2005) suggest only limited competition. In fact, except in the north, the 2 species usually show limited and unstable sympatry. Thus, except for the diffuse interactions suggested by Smith et al. (2005) in the west, and a few instances of resource overlap, there is little evidence that competition per se is a significant factor in the conservation of the northern flying squirrel.

Parasites.—A particularly intricate relationship between squirrel ecology and conservation grew out of some unusual discoveries in the southern Appalachians. In the 1960s I had set out to study the interaction of *G. sabrinus* and *G. volans* in the Appalachians as a model system for evaluating aspects of competition theory (Weigl 1968). Northern flying squirrels were exceedingly rare, but after several months of trapping I eventually captured enough for the experimental parts of my study. Colonies of both species were then housed in large outdoor aviaries in North Carolina. The 1st spring saw the demise of almost all of the *G. sabrinus* except those kept in the laboratory, whereas the *G. volans* seemed to thrive in an adjacent cage. With the help of 2 veterinarians and a former zoo pathologist, I narrowed down the cause of this massive die-off to an infection by the nematode *Strongyloides robustus*. *S. robustus* has a life cycle like that of the famous hookworms (*Necator* and *Ancylostoma*): embryonated eggs released with animal feces hatch and develop into infective larvae in the substrate; these penetrate the skin of a host, are carried to the lungs where they break through to the lumen, are swallowed, and finally lodge in the intestine doing marked physical and nutritional damage (Weigl 1968; Weigl et al. 1999). The parasite is most common in warmer climates where it has been reported to cause marked pathology in wild species (Davidson 1975). Once the cause of the affliction of the captive *G. sabrinus* was determined, other wild populations of squirrels were checked. All of the captive *G. volans* in my colony were parasitized (and were probably the source of the infection in the *G. sabrinus*), but had suffered no ill effects. In fact, all populations of *G. volans* studied in subsequent years carried this parasite. On the other hand, *S. robustus* could not be found in any of the *G. sabrinus* captured on the Appalachian peaks during the remaining years of the study. In the 1980s the federal listing of the Appalachian subspecies *G. s. coloratus* prompted a new 5-year study of the northern flying squirrel over a wide area of the North Carolina and Tennessee mountains. *G. volans* now also appeared intermittently in some of the capture sites of *G. sabrinus*, although there was never any stable sympatry of the 2 species (Weigl et al. 1999). *G. sabrinus* now supported varying intensities of parasite infection, and in the summer months there appeared to be some correlation between parasite loads and the condition of the animals (Weigl et al. 1999). We eventually cultured the parasite through its life cycle in the laboratory and determined its cold sensitivity (Wetzel and Weigl 1994) and its ability to be transferred by contact with contaminated nest material or soil substrates. Based on all the data to-date and some additional studies by Pauli et al. (2004) and Sparks (2005), I would suggest the following scenario. The cold, high-elevation or northern forests occupied by *G. sabrinus* only intermittently can support *S. robustus* because of the sensitivity of the infective larvae to cold. When *G. sabrinus* moves down into the more climatically moderate forests at lower elevations or when infected *G. volans* invade the upper slopes during the summer months along paths of human-modified habitat, the 2 species come into contact, especially by using the same tree cavities or feeding areas (Hackett and Pagels 2003), and *S. robustus* is then transferred. Even if the northern flying squirrels are not killed by the parasite, its effects may be sufficiently debilitating to put the species at a disadvantage. It is interesting that only in the colder parts of the range of *G. volans*—the Great Lakes area, northern New England, Ontario, and Nova Scotia—does one get reports of some degree of sympatry of the 2 flying squirrel species (J. Bowman, pers. comm.; Lavers 2004; Pauli et al. 2004). Why then doesn't *G. volans* take over the high-elevation refuges or northern habitats of *G. sabrinus*? The answer probably lies in sensitivity to cold of *G. volans*, its dependence on stored nuts and seeds for winter survival (Bowman et al. 2005; Doby 1984), and the virtual absence of these resources in most habitats of *G. sabrinus*. In summary, *G. volans* may possess a kind of biological weapon that at least in the southern and central part of its range, can prevent the persistence and spread of *G. sabrinus* (Barbehenn 1969; Haldane 1949; Hatcher et al. 2006; Price et al. 1988; P. D. Weigl, in litt.). It has been argued recently that the loss of genetic heterogeneity in the increasingly isolated, high-elevation populations of *G. sabrinus* of the east may make the species even more susceptible to parasite and other infections (Sparks 2005). What will happen if warming climatic conditions favor invasion of higher peaks and northern habitats by *G. volans* is thus an open question in considerations of species persistence.

Genetics

In many parts of the range of the northern flying squirrel, one can reasonably argue that the species is an island inhabitant, subject to most of the constraints that afflict other such populations (Brown 1971, 1978; MacArthur and Wilson 1967). Whether occupying real islands off the coast of Alaska; widely scattered habitats of the San Jacintos, Sierra Nevada, Rocky Mountains, and perhaps the Black Hills; or the upper elevations of the southern Appalachians, the species often occurs in small, disjunct populations, relicts of broader ranges in the late Pleistocene. The genetics of these populations have received intensive study over the last 10 years (Arbogast 1999, 2007; Arbogast et al. 2005; Bidlack and Cook 2001; Browne et al. 1999; Sparks 2005; Wartell 2005; A. Wartell, in litt.). Genetic structuring, private alleles, and loss of heterozygosity have been detected in many populations, most likely as a result of reduced population size, isolation, inbreeding, bottlenecks, and other drift effects. Although inbreeding tolerance and the replacement of alleles in time by mutation (Sparks 2005) might alleviate the plight of some groups, the loss of genetic diversity is usually seen as a potential threat, especially in changing environments. The persistence of reasonably large and interconnected populations thus appears to be critical to the species survival, and that means sufficiently large habitat reserves and the maintenance of forested corridors. Such a conservation solution might work if the environmental status quo can be maintained. However, in the face of continued forest destruction, drought cycles, El Niño effects, and the still largely unknown impacts of global climate change, the reduction of available habitat and of corridors could well spell the regional demise of this species from both a loss of genetic variability and the loss of viable places to live.

THE IMPACT OF HUMAN ACTIVITY

Habitat Size and Quality

So far I have emphasized some of the complexities of northern flying squirrel ecology and its implications for species conservation. However, it is clear that the really major threats to these squirrel's persistence come from human activities, especially in areas of small disjunct populations such as those on islands or at the southern extension of the range. Clearcutting, development, or anything that destroys extensive tracts of habitat will have obvious harmful effects. The size of the remaining forest habitat and its condition then becomes critical to survival. One has only to fly over parts of the Rocky Mountains, Sierra Nevada, and Cascades or along the Appalachians to appreciate the scope of forest destruction and roadway construction in national and privately owned forests. And landscape modification is not the only concern. Successional and regenerating communities require considerable time to develop into habitats of sufficient quality to support flying squirrels. Using demographic models, Smith and

Person (2007) have questioned the adequacy of the size of planned reserves in Alaska; Carey and others (Carey 1995; Carey et al. 1999) have provided evidence that the 2nd-growth landscapes of the Pacific Northwest do not always have the same capacity as old growth for supporting flying squirrels. In the Sierra Nevada, thinning and controlled burning may have adverse impacts on the canopy and organic material on the ground, respectively. Finally, some 2nd-growth stands may well appear to support healthy densities of squirrels, but, in reality, are population sinks for migrants from neighboring old-growth habitats and thus may not permanently maintain viable populations (Smith and Person 2007). Only long-term studies can provide the conclusive data on the suitability of these special or successional areas. The small disjunct squirrel populations of the central and southern Appalachians appear particularly vulnerable to any further modification or reduction of their habitats.

Given the above problem of loss of quality habitat, one needs to recognize 2 major forces that can aggravate this threat. One is economic and political—the demand for forest products and recreation venues, for local and regional employment, and for tax revenues and investment returns. These factors are of overwhelming significance, but are beyond the scope of this paper. The other force—climate change—is more intangible. A warming climate could cause the retreat of some tree species and communities to higher latitudes and cause the substantial reduction or elimination of boreal communities on mountains. Change in the composition and the position of communities might be especially dire in areas already modified by other human influences. Thus, the persistence of northern flying squirrels in the already-disturbed forests of West Virginia could be more tenuous than many have thought during a period of global warming. In addition to modifying community composition and distribution, climate change may have another major impact. A recent paper by Westerling et al. (2006) has documented a link between progressive climate warming and changes in the phenology, desiccation, and fire frequency in western forests. Thus, climatic warming may not only cause modifications of forest distributions, but also their complete annihilation by fire. It is likely that the desiccation observed by Westerling et al. (2006) would also have a marked impact on the moisture-requiring staple foods (fungi and lichens) of flying squirrels.

Habitat Connectedness

Along with habitat size and quality, habitat connectedness assumes an important role in species preservation. The extent of unsuitable terrain between high-quality habitat and the absence of wooded corridors could be major factors in regional survival. Frequently, the greater the reduction of contiguous forest, the wider the barriers to dispersal. Such fragmentation of flying squirrel distributions could destroy the viability of metapopulation-structured groups of squirrels, and the resulting small isolates then would be susceptible to the genetic problems mentioned earlier.

The impact of barriers on movements of flying squirrels needs further study, especially the effects of the proliferation of roadways through quality habitats. One example of barrier effects comes from the southern Appalachians. A 3-year study of an extravagant economic development scheme in the North Carolina–Tennessee mountains called the Cherohala Skyway revealed such unexpected impacts (Weigl et al. 2002). Clearly, a 2-lane scenic road removes a quantity of habitat, but, of greater significance, it also can act as a barrier to dispersal to different parts of the forest. Although *G. sabrinus* is an able glider and is known to cover distances along the ground, it is unable to cross wide, exposed roadways, especially the kind of blast-and-fill rights-of-way commonly cut into the sides of mountains. In 2 years of telemetry and trapping, no squirrel was observed to have crossed the Cherohala Skyway. The resulting range fragmentation may doom this southernmost population. In addition to barrier formation, there are 2 more-subtle impacts from a roadway. One impact was detected in the winter when snow permitted the identification of mammals moving on or along the roadway. It was obvious that various predators—bobcats, coyotes, and foxes—used the roadway as patrol routes when hunting and might easily catch any small mammals on the road. Hawks and owls also hunted over the road. Thus, one can easily see that such a right-of-way is both a physical barrier and a site of increased mortality. Another effect of roadways or similar corridors is the modification of adjacent vegetation or other habitat conditions in ways that favor the invasion of potential predators, competitors, or pathogens. In the case of *G. sabrinus*, strips of oak, cherry, and other hardwood species in disturbed areas along roadways provide foods for *G. volans* and favor its invasion of high-elevation habitats, and the transfer of *Strongyloides* to *G. sabrinus*. Thus, linear disturbances of a certain width and severity are a potential source of species fragmentation and possibly increased deleterious species interactions. The impact of roads, systems of ski trails, ridge-top wind farms, recreational vistas, and other types of habitat subdivision need careful evaluation in the future—much more then they have received to-date.

Pathogens, Pests, Pollutants, and People

Another anthropogenic factor threatening northern flying squirrels is the introduction of plant pathogens, insect pests, and industrial contaminants into squirrel habitats. In the southern Appalachians, the high-elevation conifer forests have been decimated by an adelgid insect (*Adelges piceae*) that kills Fraser fir (*Abies fraseri*), a valuable timber and Christmas tree species and a source of food and habitat for northern flying squirrels (Amman 1966; Amman and Speers 1965). The staminate cones of fir and spruce are important foods for flying squirrels in the spring when they are eaten in vast quantities. Interestingly, both field and experimental studies suggest that the essential oils from these foods suppress gut parasites such as *Strongyloides* (Weigl et al. 1999). The loss of Fraser fir then would remove a source of food (truffles, staminate cones, and possibly seed), den sites, and a possible natural medicine. In any case the adelgid killing firs, a new adelgid now destroying hemlocks, the impact of pine bark beetles in some parts of the west, and the effect of acid precipitation on vegetation and soils all represent potential threats to flying squirrels.

The last intrusion mentioned in the heading of this section of the text—people—usually goes unmentioned. One of the major effects of building of roads through prime habitat is the provision of access to lands for private and commercial development. The state or federal government builds a road, and nearby landowners demand the right to connect in order to develop their forest property. During an era of explosive interest in living in natural environments or in 2nd-home ownership, the demand for newly accessible forest land is intense and is often fueled by the economic aspirations of neighboring municipalities. A short trip on the Blue Ridge Parkway in the Appalachians reveals the result of this process. The end result is the loss and fragmentation of habitat and possibly a loss of flying squirrels. Thus, the inclusion of people as a factor along with pathogens, pests, and pollutants may indeed be appropriate.

SUMMARY

In the past 25 years the northern flying squirrel has come under increasing scrutiny as new studies have been initiated, papers published, and various agencies alerted to its status and ecological significance. Because of physical, logistical, and economic difficulties associated with long-term research in remote and often rugged areas, our knowledge of this species is still fragmentary, especially in the southern Rocky Mountains, parts of the Sierra Nevada, the Black Hills, and the northeastern United States. Enough is known now to form a picture of the species' ecology and those aspects of its biology that may affect its preservation. In 2 cases, the northern flying squirrel makes a positive contribution to the forests it occupies. Throughout its range its use and dispersal of mycorrhizal fungi—both hypogeous and epigeous—make it an integral part of a squirrel–fungus–tree mutualism that may well help maintain the very forests needed for its survival. In the northwestern United States and western Canada, the flying squirrel is a critical food item for the endangered spotted owl. Thus, if its habitat is protected and the squirrel is permitted to flourish, the owl has a greater probability of survival.

In spite of the fact that the northern flying squirrel is something of an ecological opportunist, versatile enough to occupy several forest types, consume a number of foods, and reproduce when conditions permit, certain of its characteristics potentially increase its vulnerability. Its dependence on fungi and lichens during much of the year confine it to a certain array of old-growth, boreal forests with cool, moist climates and abundant dead wood and organic soils. The phenology of fungi, particularly the locality and timing of sporocarp production, may require the exploitation of a multitude of widely spaced, ephemeral patches and thus the use at times of extensive home ranges or reliance on long-distance travel. In short, the area needed to support these animals may be larger than our short-term telemetry studies have indicated. And although its diet and tolerance of cold conditions facilitate survival in habitats with severe climates, the low caloric density of much of its diet may be a factor in its relatively low metabolic and reproductive rates (McNab 1986).

The influence of others animals in the environment of the northern flying squirrel needs further study. In no part of its undisturbed range does it seem adversely affected by predators or competitors. Perhaps only in human-modified areas do these markedly assume importance. In the southern and central parts of the eastern United States the possibility that the nematode *S. robustus*, carried by the southern flying squirrel, harms the northern species is unresolved. However, the obvious ability of northern flying squirrels to occupy lowland, deciduous habitats in the absence of the smaller species, their confinement to high elevations when *G. volans* is present, and the instability of populations in contact zones argue for some kind of interaction. In Ontario, Nova Scotia, and northern Pennsylvania, the 2 species have been found in the same nest boxes (J. Bowman, pers. comm.; A. Lauers, pers. comm.; M. Steele, pers. comm.), but these are areas that are climatically unfavorable for the parasite. Thus, in part of the range of the northern flying squirrel a parasite-mediated interaction may be operating. Clearly more research on this topic is needed.

Although there is abundant evidence of the effect of small population size and isolation on the genetic diversity of northern flying squirrel populations, there is at present no evidence of a direct link between loss of genetic diversity and survival. The isolation of populations may occur naturally because of climatic responses of forest communities, but, more likely today, it is caused—or least aggravated—by human activity. We may never know when genetic impoverishment is a major or just a contributing factor to a population's disappearance.

All of the above ecological aspects of the biology of the northern flying squirrel may have varying effects on the perpetuation of populations in different parts of the range. When one adds the human component, the probability of survival can change spectacularly. Human influences on habitat size, quality, and connectedness are most likely the main threats to the species throughout its range. These critical factors in turn are the products not only of direct habitat destruction and modification, but indirect effects such introduced pathogens, pests, and contaminants and the slow, inexorable pressure of climate change. Survival of the species *G. sabrinus* is certainly critically dependent on an understanding of the species' ecology, but, even more important, an awareness of the impact of human activity on this ecology throughout its range.

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Special Feature Editor was Barbara H. Blake.

Attachment 16

A New Green Salamander in the Southern Appalachians: Evolutionary History of *Aneides aeneus* and Implications for Management and Conservation with the Description of a Cryptic Microendemic Species

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A New Green Salamander in the Southern Appalachians: Evolutionary History of *Aneides aeneus* and Implications for Management and Conservation with the Description of a Cryptic Microendemic Species

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Green salamanders (Plethodontidae: *Aneides aeneus*) are rock outcrop habitat specialists, possessing numerous unique morphological adaptations (e.g., prehensile tail and squared toe-pads) for climbing. Some authors believe *A. aeneus,* which is widely distributed across the Appalachian Mountains of the inland eastern United States, comprises a species complex due to substantial karyotypic variation among populations. We conducted a population genetic and phylogenetic study across the range of *A. aeneus* and discovered substantial genetic structure, including four distinct lineages, one of which we describe as *Aneides caryaensis,* new species. Restricted to a narrow geographic distribution in western North Carolina, this species faces pressing conservation threats due to rapid real estate and tourism development in the area. We also recommend the recognition of three geographically distinct and reciprocally monophyletic lineages as evolutionarily significant units due to strong mitochondrial and nuclear differentiation among them. *Aneides aeneus* has been petitioned for listing under the Endangered Species Act, and our study further highlights the need for conservation management of this complex. Our formal recognition of the extent of genetic and evolutionary diversification of the complex is a critical step in establishing conservation strategies.

THE global decline of amphibians as a result of rapid growth of human populations and their expansive impact on natural environments is well documented (Petranka, 1998; Collins and Storfer, 2003; Becker et al., 2007), with many species facing rapid declines and extinction (Pechmann and Wilbur, 1994; Alford and Richards, 1999; Stuart et al., 2006; Wake, 2012). A major challenge in worldwide amphibian conservation efforts is that many amphibian species are characterized by a highly conserved morphology (Cherty et al., 1978; Wake, 1991; Stuart et al., 2006; Kozak and Wiens, 2010). This morphological conservatism in turn exacerbates the challenge of delimiting species, thus preventing meaningful assessment of their true conservation status. Inaccurate or incomplete taxonomy may exacerbate population declines and elevate extinction risks through the lack of necessary conservation interventions.

Species delimitation is one of the most difficult and controversial subfields of ecology and evolutionary biology (De Queiroz, 2007; Wiens, 2007), yet given trends in defaunation (Dirzo et al., 2014), the need for characterizing extant diversity is growing. Modern molecular tools, a more unified species concept (i.e., the generalized lineage concept; De Queiroz, 2007), and tree-based delimitation methods have led to the identification of a plethora of unique taxa from cryptic complexes, establishing them as invaluable tools in this endeavor (Bogart and Tandy, 1976; Hillis et al., 1983; Highton et al., 1989; Wynn and Heyer, 2001; Jockusch and Wake, 2002; Pauly et al., 2006; Elmer et al., 2013).

A common approach to facilitate species delimitation is to leverage phylogenetic species delimitation methods (e.g., Roy et al., 2014; Kotsakiozi et al., 2018; Titus et al., 2018). Based on, in part, the phylogenetic species concept, these methods use phylogenetic and coalescent models to guide the delimitation of species from empirical phylogenies (e.g., Pons et al., 2006; Yang and Rannala, 2010; Zhang et al., 2013; Yang, 2015). However, methods leveraging the multispecies coalescent have recently been demonstrated to often delimit population structure, not species (Sukumaran and Knowles, 2017). To avoid this concern, at least in part, methods reliant on models other than the multispecies coalescent may be used (e.g., Poisson Tree Processes [PTP; Zhang et al., 2013]). Species delimitation necessitates the use of multiple lines of evidence (e.g., molecular, morphological, ecological, and biogeographical). Although no single phylogenetic species delimitation method is invulnerable to the concerns voiced by Sukumaran and Knowles (2017), these approaches serve as a valuable complement to other sources of evidence when describing cryptic diversity.

Cryptic salamander complexes frequently occur in areas that contain a rich variety of climatic zones, geologic formations, and habitat diversity (e.g., California Floristic Province [Myers et al., 2000; Lapointe and Rissler, 2005; Rissler et al., 2006; Rissler and Apodaca, 2007; Reilly and Wake, 2015] and eastern and southern Mexican highlands [Rovito et al., 2013]). The Appalachian Mountains of the eastern United States display many of these attributes and are one of the oldest continuously exposed land masses on earth. They have a rich geologic history (Pickering et al., 2003), a diverse set of climatic regions, and a multitude of rare ecosystem types, many of which are globally threatened (Noss et al., 1995). The southern Appalachians are especially rich in biodiversity and endemism (Pickering et al., 2003) and are a center of diversity for many taxa.

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Notably, salamanders of the family Plethodontidae are extremely diverse in this region and have high levels of both endemicity and cryptic diversity (Camp and Wooten, 2016). A classic example of non-adaptive radiation (Rundell and Price, 2009), salamanders of the genus *Plethodon* diversified rapidly in the eastern United States, yet are extremely morphologically conserved (Kozak et al., 2006). Hypothesized to be driven by phylogenetic niche conservatism (Kozak and Wiens, 2006), this radiation has produced multiple cryptic species that have been delimited, at least in part, using molecular data (e.g., Highton, 1995, 1999; Highton and Peabody, 2000; Kuchta et al., 2018).

The identification of cryptic species is especially important for species that are experiencing rapid population decline. One such susceptible species is the green salamander (*Aneides aeneus*), which is listed as Near-threatened with decreasing populations by the IUCN Red List (Hammerson, 2004), Vulnerable by NatureServe (Hammerson and Dirrigl, 2017), and has been petitioned for listing under the federal Endangered Species Act. Note, however, that both the IUCN and NatureServe threat categories are in need of revision, having last been assessed 14 and 11 years ago, respectively. Consequently, these threat categories likely do not reflect the present-day population trends.

Aneides aeneus is a habitat and microhabitat specialist, making the species particularly vulnerable to habitat loss and fragmentation. These salamanders are generally associated with crevices and hollows of rock outcrop, woody, and arboreal habitats within cove forests (Gordon, 1952; Cupp, 1991; Waldron and Humphries, 2005; Smith et al., 2017). These scansorial animals are frequently found in shady crevices in rock outcrops in mixed mesophytic forests from ~300 to 1200 m elevation. Although they have been observed to exhibit arboreal tendencies, they typically are restricted to areas harboring rock outcrops (Gordon, 1952; Waldron and Humphries, 2005; Smith et al., 2017). A large number of nesting sites have been detected on rock faces (but see Pope, 1928), which may in turn lead breeding populations to be patchily distributed (Petranka, 1998; Corser, 2001). In the 1970s, green salamander populations collapsed in the disjunct Blue Ridge Escarpment (Snyder, 1983, 1991; Corser, 2001). However, those outside of the escarpment within the western portion of their range remained stable (Snyder, 1991). Corser (2001) studied trends in several populations in the Blue Ridge Escarpment throughout the 1990s and found a 98% decline in relative abundance since 1970; extensive surveys over the last 30 years by the North Carolina Wildlife Resources Commission (NCWRC) indicate that declines appear to have continued in these populations over the last decade and a half.

In this study, we examined spatial genetic patterns in *A. aeneus* through the integration of population genetic and phylogenetic methods. We analyzed 12 polymorphic microsatellite loci from specimens collected from across the range of *A. aeneus* in western North Carolina, including samples obtained from Virginia to represent the northern Appalachian portion of their range. In doing so, we sought to define geographic patterns of genetic variation and population structuring across the range of *A. aeneus* in western North Carolina. We additionally conducted phylogenetic analyses using 1) targeted mitochondrial sequences (Cyt *b* and 12S rDNA) of 74 samples across the range of *A. aeneus* and 2) a nuclear multilocus SNP dataset for ten individuals across the range obtained using 3RAD-seq (Bayona-Vásquez et al., 2019: bioRxiv:205799). Population assignments obtained using microsatellites are concordant with the identification of clades in the phylogenetic component of this study. Preliminary morphological comparisons among near-topotypic *A. aeneus* and the clade restricted to the Hickory Nut Gorge (HNG) of western North Carolina reveals cryptic, but unequivocal differentiation that is concordant with molecular evidence. As such, we describe this lineage as *Aneides caryaensis,* a new species, the first cryptic species to be described within the subgenus *Castaneides* (eastern *Aneides*: Dubois and Raffaëlli, 2012).

MATERIALS AND METHODS

Population sampling.—For the microsatellite analysis, we collected tail tips (<5 mm) from each specimen and immediately preserved them in 95% ethanol. We then stored these in a –70°C freezer at Tangled Bank Conservation until extraction. We sampled in the Blue Ridge Escarpment (BRE: 197 samples) and Hickory Nut Gorge (HNG: 28 samples) areas of western North Carolina. An additional 17 samples collected from western Virginia were donated to the study by the University of Virginia's College at Wise and were stored in a like manner. In total, 242 samples were collected for microsatellite analysis.

For analysis of mtDNA, we employed an iterative strategy of geographically dispersed sampling, analysis, targeted re-sampling, and re-analysis (e.g., Jockusch and Wake, 2002; Morando et al., 2003; Moritz et al., 2009). From the late 1990s to the middle 2000s, we collected tail clippings from specimens at 72 different populations (74 total samples) and immediately placed them in 95% ethanol and maintained them at –80°C until extraction.

Microsatellite discovery.—Total genomic DNA was extracted from eight individuals using Qiagen DNeasy kits (Qiagen, Valencia, CA) according to the manufacturer's protocols. An Illumina paired-end shotgun library was then prepared and sequenced on an Illumina HiSeq. Resulting reads were analyzed using the program PAL Finder v0.02.03 (Castoe et al., 2012). This program extracted reads containing di-, tri-, tetra-, penta-, and hexanucleotide microsatellite repeats. Resultant positive reads were then analyzed with Primer3 (version 2.0.0; Koressaar and Remm, 2007; Untergasser et al., 2012) to design primer sequences. Only those loci with sequences that were identified once or twice in our total set of reads were selected for optimization in order to avoid primer sequences that may amplify in multiple locations across the genome.

Primer testing and validation.—From the set of putative microsatellite loci described above, we selected 48 loci for amplification and polymorphism across 20 individuals from three populations according to the protocol described by Eschbach and Schöning (2013). This protocol does not necessitate individual genotypes. Instead, presence/absence of alleles in pooled DNA samples are used to estimate within and among population variability in allele frequency. Loci were then scored according to the degree of this variation using the scoring procedure described by Eschbach and Schöning (2013).

Multiplex PCR reactions were conducted using Qiagen Multiplex PCR kits (Qiagen, Valencia, CA) in accordance to the manufacturer's protocols, albeit conducted in 10 μL volume reactions. Each locus was amplified individually using labeled primers. PCR cycles consisted of an initial 15

Table 1. Results of the analysis of molecular variance (AMOVA).

Source of variation	Percent variance	*P*-value
Within population	0.712	
Among population	0.288	0.001

min denaturing step at 95°C, followed by 35 cycles of 30 sec denaturing at 94°C, 90 sec annealing step at 57°C, and a 60 sec extension at 72°C. A 30 min extension step at 60°C followed the 35 cycles, prior to cooling the product to 4°C. Resultant amplification products were then sized on a ABI 3730 DNA Analyzer at Florida State University using a GS500HDROX size standard. Results were then scored using Geneious version 6.1.6 (Kearse et al., 2012). Of the 48 loci screened, 12 were sufficiently polymorphic within and among populations and amplified consistently with easily scored genotypes (Table 1).

Microsatellite characterization.—With the 12 polymorphic microsatellite loci identified using the method of Eschbach and Schöning (2013), we then validated these loci by genotyping the 242 samples collected for this study. Procedures for amplification, genotyping, and scoring of these samples was the same as previously described. Once scored, observed (H_O) and expected (H_E) heterozygosities and number of alleles per locus (k) were calculated using GenoDive v2.0b25 (Meirmans and van Tienderen, 2004). The presence and frequency of null alleles at each locus were tested for using the R package Genepop (Rousset, 2008).

Microsatellite analysis.—To identify how populations are structured on the basis of geospatial patterns of genetic variation, we used TESS 2.3.1 (Chen et al., 2007). TESS is a program that is applied in a Bayesian framework, building Voronoi tessellations to group individuals through the identification of geographical discontinuities in allele frequencies, assuming that geographically clustered individuals will be more likely to have similar allele frequencies than more distant individuals. The number of populations (K) was estimated by running five repeat models for $K = 2$ through $K = 6$, in which 1,000,000 iterations with a burn-in of 1,000 were conducted. As suggested by François and Durand (2010), Deviance Information Criterion (DIC: Spiegelhalter et al., 2002) values were averaged between repeat runs, plotted against their respective Ks, and the value of K at which DIC plateaued was chosen as our best supported model. Populations that were defined according to this approach were then used in later phylogenetic analyses.

Once populations were defined using TESS, measures of population genetic diversity were calculated in GenoDive v2.0b25 (Meirmans and van Tienderen, 2004). Specifically, we calculated Nei's inbreeding statistic, G_{IS} (Nei, 1987), to quantify within-population genetic diversity. To quantify among-population diversity, we took two approaches. We performed a locus-by-locus analysis of molecular variance (Excoffier and Laval, 2005) using 10,000 permutations and a ploidy-independent mutation model. We then calculated pairwise values of F_{ST} using 1,000 permutations for significance, followed by a Bonferroni correction. These measures served to complement our phylogenetic reconstructions, providing an indication of the extent of gene flow occurring between recovered clades.

Targeted sequencing, alignment, and model selection.—Genomic DNA was extracted from frozen tissues using a PuregeneTM 68 kit (Gentra Systems, Inc.). Mitochondrial Cytochrome *b* (Cyt *b*) was amplified by PCR using the Cytochrome *b* primers MVZ 15 (5′–GAACTAATGGCCCA CACWWTACG–3′) and Cyt b2 (5′–CCCCTCAGAATGA TATTTGTCCTA–3′; modified from Moritz et al., 1992). 12S ribosomal DNA was amplified using primers 12SJ-L (5′–AAAGRTTTGGTCCTRRSCTT–3′) and 12SK-H (5′–TCCRGTAYRCTTACCDTGTTACGA–3′) as described by Goebel et al. (1999). Amplifications began with 4 min at 94°C followed by 45 cycles at 94°C for 1 min, 50–55°C for 1 min, 72°C for 1 min, followed by 72°C for 5 min. PCR products were purified using the GeneClean II kit (Bio 101). These products were then sequenced in both directions using a 377 ABI automated sequencer and the ABI BigDye^{TM} Terminator v3.0 Ready-Reaction Cycle Sequencing kit, but with one quarter the recommended reaction volume. Mitochondrial DNA sequences were aligned using Clustal X (Thompson et al., 1997).

Using the concatenated aligned mitochondrial sequences, we then evaluated models of nucleotide substitution using jModeltest 2.1.10 (Darriba et al., 2012). Model selection was carried out using the Bayesian information criterion (BIC: Schwarz, 1978) to evaluate the relative model fits of 88 models of evolution. A single model of nucleotide substitution was assumed for the two genes as the mitochondrion is a single non-recombining locus. Considered models included those with equal or unequal base frequencies, those that included rate variation among sites (Number Rate Categories = 6), and those with or without a proportion of invariant sites. The base tree for fitting models of nucleotide substitution was generated via Maximum Likelihood, using Subtree Pruning and Regrafting as the tree topology search operator. Cyt *b* and 12s rDNA sequences from GenBank of *A. hardii* (NC_006338) and A. *flavipunctatus* (NC_006327) were used as outgroups (Mueller et al., 2004).

3RAD sequencing and bioinformatics.—Individual extractions were normalized and prepared using a 3RAD library procedure (Adapterama III; Bayona-Vásquez et al., 2019: bioRxiv: 205799). The three enzymes used during the digestion step were BAMHI, MSPI, and ClaI. Each sample was then quadruple-indexed, limited-cycled in PCR, and cleaned using speed beads (Rohland and Reich, 2012) following the 3RAD procedure. Finally, samples were pooled together, size selected for 500 bp on a Pippin Prep (Sage Science), and sequenced on an Illumina HiSeq with a PE 150 kit (Illumina Inc.) with 5 million reads per sample. The 3RAD sequence data was demultiplexed, quality assessed, clustered, consensus called, and assembled de novo, using ipyrad v0.7.28 (Eaton and Overcast, 2016). The params file used for ipyrad is included in the supplement (see Data Accessibility).

Sequencing was done using the Illumina HiSeq 2500 platform at the University of Georgia Genomics and Bioinformatics Core. The resultant 30,524 SNPs were then filtered in VCFtools v0.1.14 (Danecek et al., 2011). Resultant SNPs following our filtering met the following requirements: minimum and maximum number of alleles per site of 2, minimum mean depth of coverage of 5, minor allele frequency of 0.2 (to remove singletons), present in at least 50% of samples. Indels were removed. Using these resultant SNPs, we produced a second SNP dataset by excluding heterozygous sites.

We produced multiple sequence alignments of these concatenated filtered SNPs in fasta format using PGDspider v2.1.1.2 (Lischer and Excoffier, 2011) and custom scripts. When converting from variant call to fasta format, PGDspider produces two concatenated sequences per sample: one generated from the concatenation of the first allele across all sites, and one from the second allele across all sites. In this way, we produced two alignments—one from the first allele at each site using the homozygous only SNPs, and one produced by concatenating the haploid, concatenated sequences of the first and second allele (using all sites) as described by Margres et al. (2018).

Mitochondrial phylogenetic reconstruction.—To infer our phylogeny using both our Cyt *b* and 12S rDNA mitochondrial sequences using BEAST 2 (Bouckaert et al., 2014), we left clock-model, site-model, and trees linked across genes as they are inherited as a single non-recombinant locus. Parameters regarding the site model (gamma shape, substitution rate, etc.) for each gene were specified according to the best-supported model from jModeltest 2; note, however, that we allowed for six gamma rate categories as the default of four may lead to excessive discretization of rate heterogeneity (Jia et al., 2014). We used a strict-clock model as our clock prior, provided the well-documented clock-like pattern of evolution of the mitochondrial genome. Provided the 0.8% pairwise sequence divergence per million years between salamandrid genera *Taricha* and *Notopthalmus* (Tan and Wake, 1995), we used a clock rate of 0.004 substitutions/site/MY $[(\frac{0.8\%}{2}) \div 100 = 0.004]$. Division by two is necessary to convert the pairwise sequence divergence to an estimate of per-branch divergence. A Yule model with a birth rate prior of 1/X was chosen to serve as an uninformative prior (Drummond and Bouckaert, 2015). All other priors were used in their default settings. The MCMC was allowed to run for 100,000,000 generations, with a burn-in of 10,000 generations, sampling every 10,000 generations.

Resulting trace files were visualized in Tracer v1.6 (Rambaut et al*.*, 2014) to estimate Effective Sample Sizes (ESS) and to check for convergence of the chain. Trees were then uploaded into TreeAnnotater v2.3.1. A burn-in of 10% was set for the annotation, with the target tree set as the maximum clade-credibility tree. Node heights of the resultant phylogenetic tree were set using the mean heights across the posterior distribution of trees. Annotated trees were then visualized using FigTree v1.4.2 (Rambaut et al., 2014).

Nuclear phylogenetic reconstruction.—SNPs obtained via 3RAD-seq were then used to produce two unrooted phylogenies, one using SVDquartets (Chifman and Kubatko, 2014) in PAUP* v4.0a157 (Swofford, 2003) as in Margres et al. (2018), and one using RAxML v8 (Stamatakis, 2014) on the CIPRES portal (Miller et al., 2010). SVDquartets uses SNPs to infer relationships among quartets of taxa under a coalescent model in which each site is assumed to have its own genealogy. Thus, the choice of concatenating each allele is compatible with this model. As input for SVDquartets, we used the concatenation of all alleles as input. In contrast, the concatenated sequence of the first allele at every homozygous site was used for RAxML.

Using SVDquartets, all quartets were estimated under the multispecies coalescent model (expecting matrix-rank 10), and these quartets were assembled using the QFM algorithm. Confidence in tree topology was quantified through nonparametric bootstrapping. A consensus tree was produced by summarizing across bootstrapped trees using the SumTrees program as implemented in DendroPy v4.3.0 (Sukumaran and Holder, 2010) using –force-unrooted and –min-clade-freq = 0.25. Consensus trees were visualized using FigTree v1.4.3 (Rambaut et al., 2014).

Using RAxML, phylogenies were inferred under the GTR model disabling rate heterogeneity, assuming each SNP evolves independently. Bootstrapping (100 replicates) was conducted using a rapid bootstrap analysis following a search for the best scoring ML tree (–f a option). The best tree and bootstrap nodal support values were visualized in FigTree v1.4.3 (Rambaut et al., 2014). We implemented the Felsenstein acquisition bias for invariant sites by specifying the number of sequenced sites not included in our final set of SNPs (Leaché et al., 2015). Because the exclusion of heterozygous sites led to the removal of a large number of sites informative to distinguishing individuals from the Hickory Nut Gorge, we also inferred a phylogeny without an ascertainment bias, thus including heterozygous sites. These results may be found in the supplement (see Data Accessibility).

Morphological materials collection.—Specimens used for this study included seven adults (3 males, 4 females) from the Hickory Nut Gorge (see below) and a series of nine adults of *Aneides aeneus* (6 males, 3 females) in the Museum of Vertebrate Zoology (MVZ) collected from SE Fort Payne along Little River Canyon, De Kalb County, Alabama (34.39°N, 85.62°W). Herein, we explicitly tested the hypothesis that specimens from the Hickory Nut Gorge differ from topotypic *A. aeneus*.

The following measurements were used for preliminary morphological comparison: snout to posterior angle of vent (standard length, SL), head width (HW), snout to gular fold (SG), head depth at posterior angle of jaw (HD), eyelid length (EL), eyelid width (EW), anterior rim of orbit to tip of snout (ES), horizontal eye diameter (ED), anterior rim of orbit to external naris (EN), interorbital distance between angle of eyes (intercanthal distance, IC), interorbital distance between eyelids (IO), snout to forelimb (SF), distance separating external nares (internarial distance, IN), snout projection beyond mandible (SP), shoulder width (SW, distance between axilla across dorsum), snout to anterior angle of vent (SAV), axilla–groin distance (AX), number of costal interspaces overlapped by adpressed limbs (negative when overlapped, positive when not overlapped [limb interval, LI]), forelimb length (FLL), hind limb length (HLL), hand width (HAW), foot width (FW), length of third (longest) toe (T3), and length of fifth toe (T5). Measurements were made using digital calipers. Tooth counts are based on direct counts of clearly ankylosed teeth. Institutional abbreviations follow Sabaj (2016). Color information was derived from photographs of living specimens.

Preliminary morphological comparative analyses.—Measurements of all 18 traits were compared among topotypic *A. aeneus* and samples from the Hickory Nut Gorge using a two-tailed Welch two-sample t-test in R version 3.4.2 (R Core Team, 2017). We omitted tail measurements from animals with regenerated tails for statistical comparisons. Additionally, to test the hypothesis that the two species are distinguishable on the basis of morphology, we leveraged both Principal Components Analysis (PCA) and Linear Discriminant Analysis (LDA). For these analyses, we excluded tail measurements, as well as counts of pre-maxillary and maxillary teeth due to the presence of missing data. We also removed costal groove counts as this character was near invariable (excluding one sample from the Hickory Nut Gorge) and thus uninformative. The remaining 14 characters were visually inspected for normality and, where necessary and possible, transformed to improve normality. Longest toe and 5th toe were log-transformed, and adpressed limbs were converted to absolute value (all values were negative) and subsequently log-transformed. Due to our low sample size, LDA was performed as follows. First, 11 of 16 samples (approximately two-thirds) were used for training, whereas the remaining five samples were to assess prediction accuracy. We then repeated this process 10,000 times, thus producing a distribution of prediction accuracies that we subsequently used to produce an estimate of mean prediction accuracy. Due to the low sample size in these analyses, we emphasize that results are preliminary in nature.

Phylogenetic species delimitation.—We chose to supplement our phylogenetic and population genetic analyses with species delimitation using Poisson tree processes (Zhang et al., 2013). The method Poisson tree processes (PTP) is not built around the multispecies coalescent, instead modeling the speciation process as a function of the substitution process. Briefly, PTP attempts to delimit the boundaries at which the substitution process transitions between the inter- and intraspecific substitution process as a function of the branching process, namely branch lengths. Notably, this method does not necessitate time-calibrated phylogenies, thus allowing us to use it with both of our molecular phylogenies. Further, the PTP has recently been demonstrated to perform with similar accuracy as both the Generalized Mixed Yule Coalescent (GMYC) and Bayesian Phylogenetics and Phylogeography (BPandP) and is more conservative than the GMYC (Luo et al., 2018).

We applied the PTP to both our mitochondrial and nuclear phylogenies on the PTP web server (https://species.h-its.org/). For each phylogeny, the analysis was run for a total of 500,000 generations, sampling every 500 generations for a total of 1,000 from the posterior. The first 10% of samples were discarded as burn-in. For the mitochondrial dataset, outgroups were retained as a reference for interspecific branching processes and to minimize false positives.

RESULTS

Microsatellite characterization.—We identified, validated, and optimized 12 microsatellite loci for use in conservation studies of subgenus *Castaneides* and other members of the genus for which microsatellites have not been developed. We found these loci to possess varying levels of diversity (Table S1; see Data Accessibility). Although the prevalence of null alleles (>10%) is not trivial (Table S1; see Data Accessibility), their presence is distributed across populations (Table S2; see Data Accessibility). However, we do not believe this to be of great concern, as null alleles have only been found to inflate, not generate, population structure or impact hypothesis testing (Chapuis and Estoup, 2007; Carlsson, 2008). Thus, the presence of null alleles in our dataset is unlikely to generate the patterns of population differentiation documented herein.

Population structuring and differentiation.—Our analysis of population structuring in TESS (Chen et al., 2007) found that $K = 4$ was the best-supported model according to the method of François and Durand (2010; Fig. S1; see Data Accessibility). From here on, these populations will be referred to as the Blue Ridge Escarpment (BRE) populations, Hickory Nut Gorge (HNG) population, and Virginia (VA) population. Of the samples, 197 were assigned to the two populations comprising the BRE, 28 were assigned to the HNG, and 17 were assigned to the VA population (Fig. 1). Interestingly, whereas K of 3 reveals unambiguous assignment of all individuals collected from the BRE to one population, the best fit model ($K = 4$) reveals apparent population structure within the BRE (Fig. 1). However, among these two populations there is appreciable admixture. Although we discovered population structuring within the BRE, individuals from VA and the HNG were always unambiguously assigned to their own populations (Fig. 1).

Table 2. Pairwise F_{ST} values for populations of *Aneides aeneus* (*Castaneides*) identified in this study. All values are statistically significant at the <0.001 level.

	BRE-1	BRE-2	HNG
BRE-2	0.076	–	–
HNG	0.289	0.305	–
VA	0.139	0.192	0.282

Results of both AMOVA (Table 1) and pairwise F_{ST} (Table 2) further revealed the extent of differentiation among populations identified by TESS. Analysis of molecular variance showed that whereas 71% of variance could be explained within populations, the remaining 29% could be explained by among-population differences ($P = 0.001$). Pairwise F_{ST} revealed that whereas the two BRE populations are little differentiated ($F_{ST} = 0.076$), the HNG population is highly differentiated from both BRE subpopulations (BRE-1: 0.289, BRE-2: 0.305) despite their close geographic proximity. Likewise, the HNG and VA populations are highly differentiated ($F_{ST} = 0.282$). Lastly, the VA population exhibits an intermediate degree of differentiation from the two BRE subpopulations (BRE-1: 0.139, BRE-2: 0.192). Importantly, all comparisons are highly significant ($P = 0.001$).

Calculations of the inbreeding coefficient (Table 3), G_{IS}, ranged greatly between populations. Notably, the first BRE population appears to be harboring an excess of heterozygosity ($G_{IS} = -0.05$, $P = 0.001$). In contrast, the second BRE subpopulation ($G_{IS} = 0.103$, $P = 0.001$) and HNG population ($G_{IS} = 0.250$, $P = 0.001$) are quite inbred, with the HNG faring particularly poorly. The VA population does not appear inbred ($G_{IS} = 0.035$, $P = 0.191$).

Mitochondrial sequence data and model selection.—Sequencing of mitochondrial loci Cytochrome *b* and 12S rDNA yielded aligned sequence lengths of 433 and 415 nucleotides, respectively. For our concatenated alignment, we found that TrN+G was the best supported model according to BIC as

Table 3. Genetic diversity and inbreeding coefficient, G_{IS}, at each population in this study. H_O is the observed heterozygosity and H_E is the expected heterozygosity within populations. Parenthetical values are the corresponding *P*-values for estimates of G_{IS}.

Population	H_O	H_E	G_{IS}
BRE-1	0.726	0.692	–0.050 (0.001)
BRE-2	0.595	0.663	0.103 (0.001)
HNG	0.455	0.607	0.250 (0.001)
VA	0.711	0.736	0.035 (0.191)

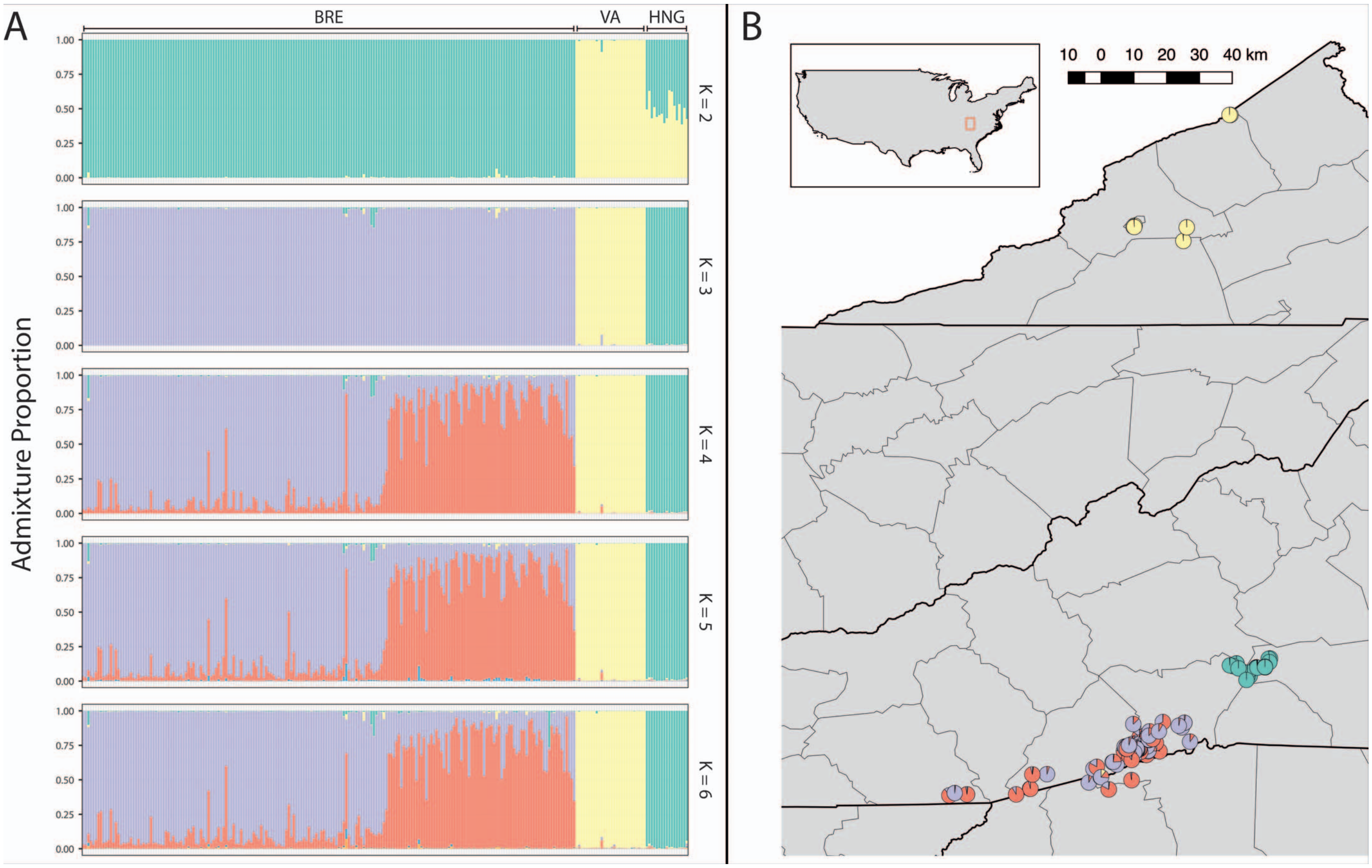

Fig. 1. Population structuring in *Aneides aeneus* (*Castaneides*) as identified using 12 microsatellite loci and TESS. (A) Results of running TESS assuming the number of populations (K) ranges from two through six. Each column represents an individual, and the colors in each bar indicate the population each individual is assigned to. The height and color of each bar represents the admixture proportion for that individual. Results for a single run are shown. (B) Representation of best-fit (K = 4) TESS results in geographic space. Samples are shown as pie charts, with colors corresponding to the populations identified in A, and the fractions corresponding to each sample's admixture proportions.

implemented in jModeltest 2. Model parameter estimates (Gamma = 0.2960, ncat = 6, Rate AC = 1, Rate AG = 4.9971, Rate AT = 1, Rate CG = 1, Rate CT = 8.9766, and Rate GT = 1.0) were thus implemented as our site model.

Phylogenetic inference.—Our Bayesian phylogenetic reconstruction using concatenated Cyt *b* mtDNA and 12S rDNA sequence data recovered four well-supported, reciprocally monophyletic lineages within *A. aeneus* (Fig. 2). Provided our strict molecular clock rate is accurate, the node heights (coalescent times) may be interpretable in terms of millions of years. Our tree thus indicates that the tMRCA (time to most recent common ancestor) between *A. aeneus* and our outgroups is roughly 30 MYA. The Hickory Nut Gorge lineage was inferred to be sister to all other lineages within the *A. aeneus* complex, with their tMRCA being estimated at around 11 MYA. The southern Appalachian lineage last shared a common ancestor with the lineage comprising the BRE and northern Appalachian lineages approximately 9 MYA. Lastly, the BRE and northern Appalachian lineages last shared a common ancestor around 5 MYA. Our estimates of divergence times are concordant with those of Shen et al. (2016: between 27.2 and 32.3 MYA), who used several methods to estimate the divergence times between *A. aeneus* and *A. hardii*.

Following SNP calling in iPyrad, a total of 30,524 SNPs were obtained. Filtering in VCFtools yielded final SNP dataset containing 3,550 SNPs across the ten samples. Reduction of these sites to only those homozygous genotypes for ascertainment bias correction reduced the number of SNPs to 2,634 in RAxML.

Using SVDquartets, four well-supported groups were identified (>95% BS support: Fig. 3A) that roughly corresponded to the four clades identified in the mitochondrial phylogeny (BRE, northern Appalachians, southern Appalachians, HNG). These findings are further substantiated by our nuclear phylogeny estimated by RAxML (Fig. 3B). This phylogeny recovered three well-supported groups (BRE, southern Appalachians, and HNG), with the northern Appalachians forming a single monophyletic group despite lower support values. Notably, the HNG and southern Appalachian lineages are separated by long branches, indicating substantial genetic divergence of each clade from the rest of the *A. aeneus* species complex. Removal of heterozygous sites led to the inference of zero-value branch lengths among samples from the HNG, a fact that reiterated the low genetic diversity harbored within this population.

Species delimitation.—We applied the PTP to the phylogenies obtained using BEAST and RAxML. Using the mitochondrial phylogeny, the PTP identified four species with a posterior support greater than 50% (Fig. 2): southern Appalachians (59%), BRE (91%), northern Appalachians (71%), and the HNG (93%). In contrast, when applying the PTP to the nuclear tree produced by RAxML (Fig. 3), we identified three species; however, support values were low overall, likely

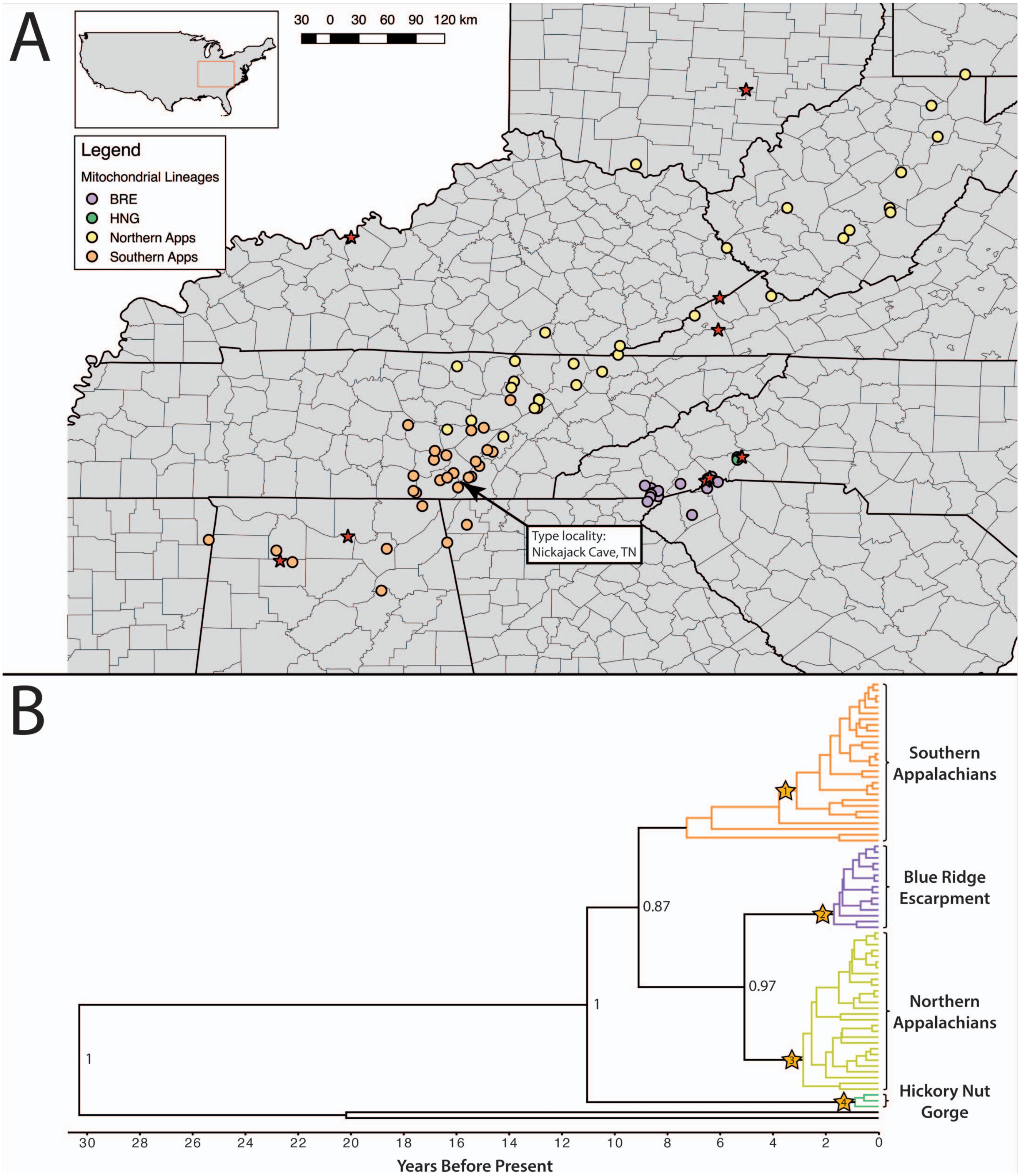

Fig. 2. Mitochondrial phylogenetic lineages within *Aneides aeneus* (*Castaneides*). (A) Localities of samples used in the mitochondrial phylogenetic analysis. The type locality (Nickajack Cave) is in Tennessee, at the border with Alabama; specimens from this vicinity are nested in the southern Appalachian clade. Red stars are locations used in the nuclear phylogenetic reconstruction. (B) Bayesian consensus tree of Cytochrome *b* and 12S rDNA sequences as produced by BEAST 2. Node labels represent posterior probabilities for the four main lineage splits within *A. aeneus* (*Castaneides*). Outgroup contains one voucher specimen each of *A. hardii* and *A. flavipunctatus*, obtained from GenBank. Numbered stars indicate clades identified by PTP to comprise unique species across the majority of the posterior distribution. Support values for clades are as follows: 1) 59%, 2) 91%, 3) 71%, 4) 93%. See Data Accessibility for tree file.

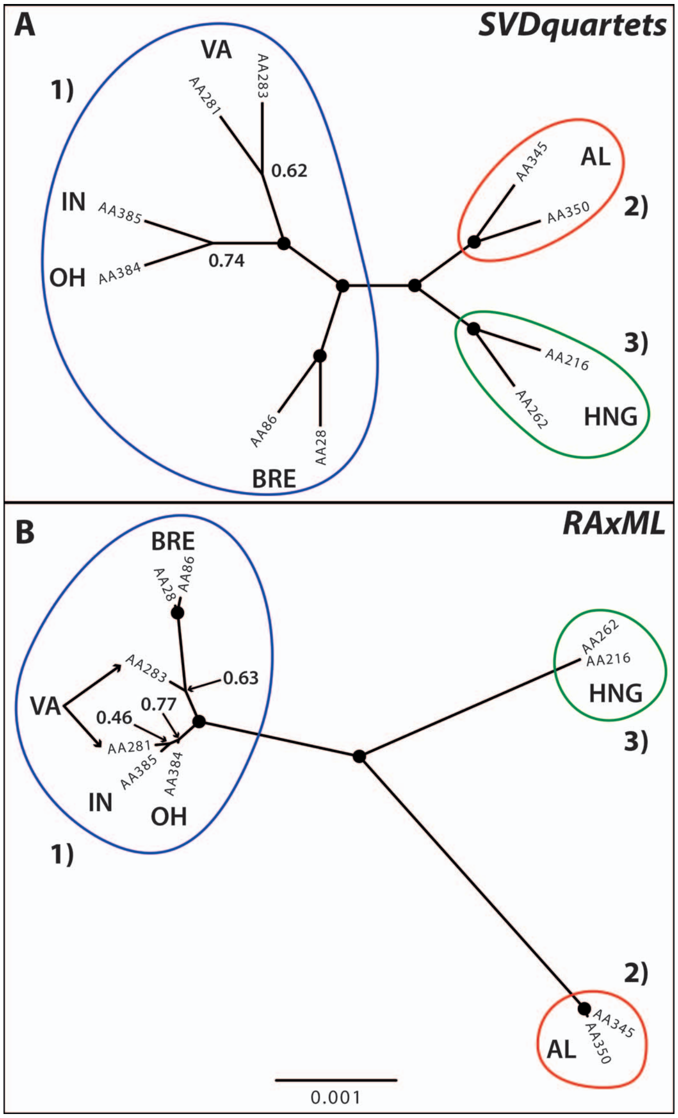

Fig. 3. Nuclear phylogenies as estimated by (A) SVDquartets and (B) RAxML. Filled circles denote nodes with support values >95%, empty circles denote nodes with support values >90%, and all other nodal support values are indicated as text. Numbered/colored circles enclosing monophyletic groups in the trees indicate species as identified by PTP when applied to the best tree identified by RAxML. Support for these species are as follows: 1) 44%, 2) 82%, 3) 50%. Low support values for the HNG are due to zero branch lengths (see Fig. S4; see Data Accessibility). Corresponding circles are similarly shown on the tree inferred by SVDquartets. However, PTP was not applied to this phylogeny as SVDquartets does not yet infer branch lengths. See Data Accessibility for tree files.

owing to the low number of substitutions observed within species using the reduced dataset for RAxML (excluding heterozygous sites). Specifically, we recovered the following as species: southern Appalachians (82%), northern Appalachians/BRE (44%), and the HNG (50%). The low support for the HNG is almost certainly a consequence of there being zero-value branch lengths separating the two samples. This limits the ability of PTP to test hypotheses that the substitution process differs within and among species. Analysis of a phylogeny inferred by RAxML without an ascertainment bias (and thus including heterozygous sites) confirms this. Doing so, we identified three species with support values greater than 50% (Fig. S4; see Data Accessibility): southern Appalachians (97%), northern Appalachians (57%), and the HNG (95%).

Species description.—Herein we describe the Hickory Nut Gorge lineage as a distinct species on the basis of molecular and preliminary morphological differentiation. We adopt the use of subgenus *Castaneides* (Dubois and Raffaëlli, 2012) when referring to the species complex of green salamanders, as this etymology was initially established to refer to eastern *Aneides* which was thought to harbor at least three cryptic species.

Aneides (Castaneides) caryaensis, new species

urn:lsid:zoobank.org:act:99D016DA-6505-4787-BEEB-430C9834F530
Hickory Nut Gorge Green Salamander
Figures 4, S5; Tables 4, S3

Holotype.—NCSM 33389, adult male, USA, North Carolina, Rutherford County, from near Bat Cave (exact location withheld due to conservation concerns), 537 m elevation, J. R. Bailey, 22 June 1962.

Paratypes.—Herein, exact location data for populations other than the holotype of *A. aeneus* are withheld due to conservation concerns. USNM 446474, same data as holotype; MVZ 178584–178585, North Carolina, Henderson County, A. Westerman, 1 October 1981; NCSM 33389–33391, North Carolina, Rutherford County, just E. of Henderson Co. line on north side of river, across from Bat Cave, near Bat Cave (ca. 1.9 air miles WNW Chimney Rock), J. R. Bailey, 22 June 1962.

Diagnosis.—A member of the clade and genus *Aneides,* subgenus *Castaneides*, distinguished from the only other member of the subgenus, *A. (C.) aeneus,* by DNA sequence and preliminary morphological differences. Broadly similar in morphology to A. *aeneus* but differing in some aspects of coloration and in having 1) broader and elongated heads, 2) a greater number of maxillary and premaxillary teeth, 3) limbs that are slightly longer in relation to size, 4) broader shoulders, and 5) broader feet and longer toes.

Description.—Slender, very long-legged species of moderate size (maximum known standard length, SL, female 59.8 mm) with slender, whip-like tail (0.93–1.07 SL; longest in male holotype for which tail appears to have regenerated very early in life) and very long digits (longest toe 4.4 mm). Standard lengths (SL) of three sexually mature males are 48.5, 52.4, and 58.4 mm; four females are 52.8, 57.8, 58.9, and 59.8 mm. Head and body are strongly flattened; legs typically extended directly lateral from body. Long limbs overlap by 2.5 to 4 costal interspaces when adpressed to the sides of trunk. Head relatively broad (0.17–0.19, mean 0.18 SL); adductor muscles of jaw bulge outward behind eyes, slightly more in males than in females. Eyes large and prominent. Premaxillary teeth slightly enlarged in relation to those of plethodontids excluding other *Aneides*; in males teeth penetrate upper lip. Maxillary teeth are in short row; posterior portion of maxillary bone is edentulous (as in fig. 2 of Wake, 1963). Anterior vomerine teeth in concave rows of medium length. Posterior vomerine teeth numerous, organized into unified patch on the roof of mouth. Mandibular teeth borne on dentary bone include two to five very large,

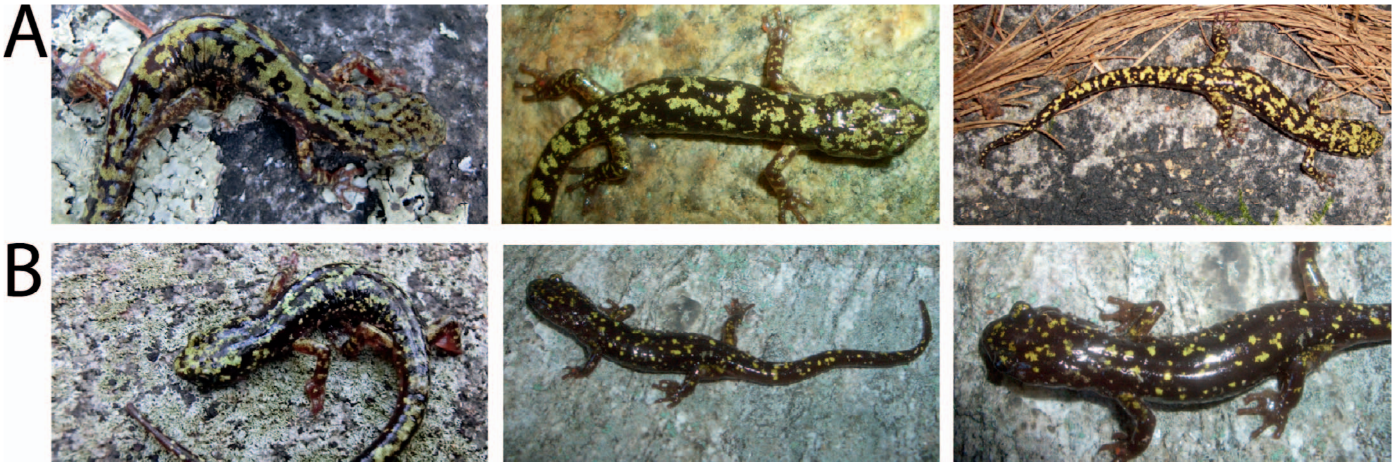

Fig. 4. Images representing *Aneides aeneus* (A) and *A. caryaensis* (B). *Aneides caryaensis* is characterized by smaller and less connected lichen-like patches of bright green to yellowish-green pigment.

conical, recurved teeth; other teeth small to moderate in size. Premaxillary teeth number range from three to seven in males, five to 17 in females. Maxillary teeth totals are 12 to 15 in males, 11 to 24 in females. Very small anterior vomerine teeth totals range from six to 17 in males and 12 to 19 in females. Only potential sexual dimorphism noted is somewhat higher tooth numbers in females and slightly larger jaw muscles in males.

Measurements (in mm), limb interval, and tooth counts of the male holotype.—SL 58.4, TL 62.6, HW 10.7, SG 15.0, HD 4.5, EL 3.5, EW 1.9, ES 3.8, ED 2.7, EN 3.1, IC 5.6, IO 3.4, SF 20.6, IN 3.0, SP 1.1, SW 6.6, SAV 53.8, AX 29.2, LI –4, CG 14, FLL 17.4, HLL 21.0, HAW 6.7, FW 8.8, T3 4.4, T5 3.2, TP 1.1, TW 3.6, TD 3.2. No mental gland evident. MT 8–7, DT 8 (4 large)–6 (5 large), VT 6–9, PMT 3.

Coloration in life.—Ground color of dorsal surfaces is dark brownish-black. Dorsum covered by lichen-like patches of bright green to yellowish-green pigment. Flanks do not have dark pigment as in *A. aeneus*; instead have light grayish-yellow ground color on flanks and venter with loose suffusion of punctate melanophores. Density of punctate melanophores decreases on venter, especially near midline, so venter appears much lighter than other surfaces.

Comparisons.—Six adult males range from 49.9 to 55.3 mm SL (mean 52.8), similar to the three males of the new species (48.5–58.4, mean 53.1 mm); three adult females ranged from 44.8 and 53.0 mm (mean 49.5 mm), compared with the somewhat larger females of the new species (52.8–59.8, mean 57.3 mm). Some additional individuals from the Fort Payne area are mature at even smaller sizes; mental glands are present on males as small as 47.3 SL; a specimen from near

Table 4. Means of untransformed morphological characters for each *Aneides aeneus* and *A. caryaensis*. Sample sizes are indicated for each species, as are the results of a two-tailed t-test comparing means among species assuming heteroscedasticity. As with our analysis using PCA and LDA, data were normalized where possible and necessary (longest toe, 5th toe log transformed; adpressed limbs converted to absolute value and then log transformed). Units are specified below the character being measured. Standard deviations are reported alongside means. Comparisons were made among untransformed characters.

Trait	*Aneides aeneus* ($n = 9$)	*Aneides caryaensis* ($n = 7$)	T-test *P*-value
Snout to vent (mm)	51.70±3.02	55.51±4.27	0.072
Axilla to groin (mm)	25.98±1.62	27.16±2.11	0.247
Head width (mm)	8.69±0.74	10.01±0.83	0.006
Forelimb (mm)	14.53±0.88	16.21±1.09	0.006
Hindlimb (mm)	17.08±1.4	18.84±1.42	0.027
Longest toe (mm)	3.39±0.27	3.87±0.42	0.018
5th toe (mm)	2.36±0.11	2.89±0.37	0.007
Costal grooves (count)	14.00±0	14.14±0.38	0.356
Adpressed limbs (# costal interspaces overlapped by adpressed limbs)	–2.11±0.33	–3.36±0.56	<0.001
Snout to gular fold (mm)	12.77±0.88	14.07±0.79	0.008
Tail (mm)	54.14±5.22	56.08±5.45	0.586
Premaxillary teeth (count)	5.56±2.01	9.17±4.31	0.048
Maxillary teeth (count)	9.00±2.65	15.67±4.97	0.005
Vomerine teeth (count)	12.44±3.24	14.14±4.45	0.575
Foot width (mm)	6.91±0.54	7.96±0.82	0.016
Shoulder width (mm)	5.46±0.5	6.50±0.63	0.004
Eye–nostril (mm)	2.67±0.15	2.97±0.17	0.003
Eye–snout (mm)	3.69±0.39	4.07±0.39	0.071

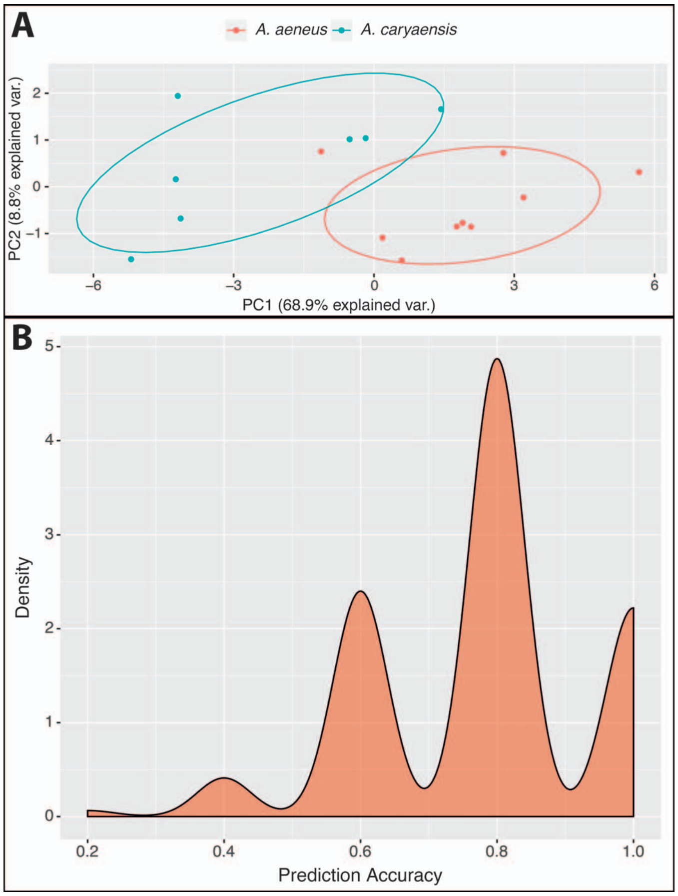

Fig. 5. Results of Principal Components Analysis (PCA) and Linear Discriminant Analysis (LDA) using 14 morphological characters. For both analyses, data were normalized where possible and necessary (longest toe, 5th toe log-transformed; adpressed limbs converted to absolute value and then log-transformed). (A) PCA with points and normal data ellipses colored by species. (B) Density plot of prediction accuracy by LDA across 1,000 permutations of samples for the training and prediction sets.

Bat Cave has male cloacal morphology at 43.9 SL but no mental gland. These two populations are both near the southern limit of the genus in eastern North America. It thus appears that southern populations of *Castaneides* are smaller, on average, than more northern ones because *A. aeneus* is reported to reach 140 mm total length (the species occurs as far north as south-central Pennsylvania (39.97°N) and as far south as west central Alabama (at least to 32.75°N; Petranka, 1998; AmphibiaWeb, 2018). Juterbock (1989) reports males as large as 65.5 SL and females 65 SL in extreme southern Ohio.

The limbs are relatively longer in *A. caryaensis* relative to *A. aeneus*; combined limb length is 0.63–0.67 SL (mean 0.65) in males and 0.61–0.62 SL (mean 0.62) in females, compared to 0.60–0.65 SL (mean 0.62) in males and 0.58–0.62 SL (mean 0.60) in females of the Fort Payne sample of *A. aeneus*. Additionally, the adpressed limbs cover 2.5 to 4 costal folds in the new species, in contrast to 1.5 to 2.5 in the Fort Payne (*A. aeneus*) sample. All specimens of the new species and of our Fort Payne sample have 14 costal grooves, which means that they have a high likelihood of having 15 trunk vertebrae. Wake (1963) reported counts of 15 (two specimens), 16 (10 specimens), and 17 (4 specimens) trunk vertebrae in individuals from across the range of *A. aeneus*.

Numbers of teeth are broadly similar in the two species. In *A. aeneus*, number of premaxillary teeth ranges from 3–9 and number of vomerine teeth ranges from 8–19. In *A. caryaensis,* number of premaxillary teeth ranges from 5–17 and number of vomerine teeth ranges from 6–19. Number of maxillary teeth differs among species, with *A. aeneus* possessing between 5 and 13, whereas *A. caryaensis* possesses between 11 and 24. This increase in the number of maxillary teeth in *A. caryaensis* relative to *A. aeneus* corresponds to a lengthening and widening of the head (Table 4).

Aneides caryaensis is morphologically cryptic in relation to *A. aeneus* according to our preliminary analyses. However, there exist several notable differences among the two species (Table 4; Fig. S5; see Data Accessibility). Outside of coloration differences (Fig. 4), *A. caryaensis* possesses wider and longer heads (perhaps associated with more hypertrophied jaws), longer toes, as well as wider feet and bodies (Table 4). In summary, *A. caryaensis* appears broader, albeit not significantly longer in total length (Table 4). Notably, both PCA and LDA indicate that the two species are morphologically differentiated (Fig. 5) using our preliminary data. A great amount of variance (68.9%) was explained by PC1, with an additional 8.8% explained by PC2 (Fig. 5A). No one variable loaded most heavily onto PC1, however. Our permutation procedure for LDA revealed that we are able to distinguish among species, on average, with 79% prediction accuracy (Fig. 5B).

Habitat and geographic range.—Observations recorded by the collector of the holotype, Joseph R. Bailey (field notes stored in North Carolina State Museum) include the following: "June 22, 1962.—about 5–9 PM. Weather warm and fair. Conditions fairly moist." Specimens collected "just above road on north side of [Broad] river in tight rock crevices in late afternoon. Only a few suitable crevices here. One individual taken at night from rock face on opposite side of river in [*Plethodon*] *longicrus* area. When tickled into sack below it, it flattened against rock and it was necessary to pry it off in contrast to *longicrus* of same size which would drop off."

Co-occurring species.—Three other plethodontid salamander species are commonly encountered in syntopy with *A. caryaensis*: *Plethodon amplus* (*Plethodon jordani* species complex), *Desmognathus cf. carolinensis* (possible intergrade with *Desmognathus ocoee*), and a species that occurs in microsympatry and is currently assigned to *Plethodon yonahlossee*, but that was described originally as *Plethodon longicrus* (Adler and Dennis, 1962). Importantly, the type locality of *A. caryaensis* (the Hickory Nut Gorge) is also the type locality of *P. longicrus*. It is noteworthy that these three species are either endemic to the range of *A. caryaensis* or are differentiated to the extent that they either were once recognized as distinct taxa or are currently of undetermined status.

Etymology.—Named after the Hickory Nut Gorge of Western North Carolina to which the species is restricted. We allude to this locality by referencing the genus Hickory (*Carya*), after which the locality is named.

DISCUSSION

In this study, we integrated population genetic, phylogenetic, preliminary morphological, and species delimitation approaches to delimit cryptic species in the green salamander complex (*A. aeneus*). We find strong, consistent support for the recognition of the Hickory Nut Gorge lineage as a unique species. There is also strong support for the recognition of up to three other lineages (Figs. 2, 3). However, provided the undetermined boundaries between the northern and southern populations, which apparently overlap geographically, we recommend that these be recognized and managed as evolutionarily significant units (ESUs) for the purposes of status assessments and conservation actions, and that further research be conducted to analyze the validity of these lineages as full species.

Our phylogenies, both mitochondrial and nuclear, are concordant with previous research (Sessions and Kezer, 1987) that identifies distinct lineages within the *A. aeneus* complex. Our mitochondrial phylogenetic analysis supports the recognition of *A. caryaensis* as sister to all other members of this complex, sharing common ancestry with other lineages an estimated 12 MYA. That said, these estimates must be regarded as overestimates of the divergence times among these three species, as coalescent times (inferred in the present study) are necessarily greater than divergence times (Nielsen and Slatkin, 2013).

In addition to the strong topological support, a visualization of the geographical distribution of our samples revealed the allopatric distribution of each lineage (Fig. 2). Further, each lineage is geographically assorted with the exception of the contact zone of the northern and southern clades. Comparable levels of cryptic differentiation have also been recently delimited within formerly widespread species complexes in western *Aneides* (Reilly and Wake, 2015; Reilly et al., 2015), and, like *A. aeneus,* the ambiguous nature of these secondary contact zones in both eastern and western U.S mountain ranges seems to be driven by the complex geologic history of tectonic activity, which we discuss below. In addition to the evidence presented earlier, molecular species delimitation methods consistently and strongly support the recognition of *A. caryaensis* as a distinct species.

We find *A. caryaensis* to be sister to the *A. aeneus* complex, the product of an ancient divergence that was followed by further population subdivision in the remainder of the complex. More detailed sampling will be required to elucidate the phylogenetic history of the complex as a whole. Accordingly, in combination with our population genetic, phylogenetic, and preliminary morphological analyses, we have formally described *A. caryaensis* as a distinct species. Although populations from the Hickory Nut Gorge occur within ~25 km of populations in the Blue Ridge Escarpment, there are high levels of microsatellite differentiation (Table 2), indicating minimal gene flow. This is consistent with other members of Plethodontidae found within the Hickory Nut Gorge. For example, *P. amplus* is endemic to the Hickory Nut Gorge and a distinct form of *P. yonahlossee* that displays genetic and morphological differentiation is found within the Hickory Nut Gorge (Guttman et al., 1978; Highton and Peabody, 2000; Apodaca, unpubl. data). Formal recognition of *A. caryaensis* is urgent considering its small geographic distribution, which is estimated to be approximately 3,625 ha (~35 sq. km) and comprises fewer than 25 known localities. Further, the recent population declines (Corser, 2001) of the nearby BRE lineage and the intensifying human footprint within the Hickory Nut Gorge imply that *A. caryaensis* may be facing similar risks of decline.

***Zoogeography of* Castaneides.**—Arachnologists (Catley, 1994) have long recognized the striking similarities between salamanders in *Aneides* and syntopic rock outcrop-inhabiting relict lampshade spiders (*Hypochilus*). These ecologically comparable species (=cryophilic syndrome) share allopatric, disjunct portions of their range in California and the southern Rockies, as well as five eastern species having closely overlapping distributions with eastern *Aneides*. This includes *H. coylei* confined to the Hickory Nut Gorge, a microendemic spider described on subtle genital characters (Huff and Coyle, 1992) and molecularly upheld by Keith and Hedin (2012) as a sister clade to the Cumberland Plateau *H. thorelli*. *Hypochilus coylei* may have shared a common Miocene ancestor with a formerly more widely and easterly distributed *H. thorelli* (Catley, 1994; Hedin, 2001), and similar dynamics may have accounted for the circumscription of *A. caryaensis* within a single valley on the very edge of the southeastern Highlands. Additionally, the previously described species *P. longicrus* (now *P. yonahlossee*) and *P. amplus* are restricted to the Hickory Nut Gorge and surrounding Swannanoa Mountains as well. Although it is unclear at present whether *P. longicrus* is a distinct species or simply a color morph of *P. yonahlossee*, it seems apparent that whatever led to the diversification of *A. caryaensis* in the Hickory Nut Gorge also affected a number of other species.

We have noted that no obvious surface barrier separates the lower elevation populations of *A. caryaensis* from those in BRE, occurring just 25 km to the west (Figs. 1, 2). However, three Mesozoic-aged faults (Snipes et al., 1986) lie precisely in the region between *A. caryaensis* and the BRE clade and *H. coylei*/*H. pococki*. These lower hills are known as the Hendersonville bulge and lie just east of the Brevard fault zone—the generalized physiographic edge of the Blue Ridge province—belonging geologically to the lower relief of Piedmont Province (Hack, 1982). Rejuvenated global and Appalachian Miocene uplift 10–20 MYA (Gallen et al., 2013; Liu, 2014) altered the climate and ecosystems towards a more grassland- dominated terrestrial ecosystem at the expense of the mixed mesophytic forests (Kürschner et al., 2008). The faults are thought to have been the loci of small differential movements, most recently during the late Miocene ~12 MYA that affected the gross morphology of the terrain sufficiently to act as a barrier to gene flow for these rather sedentary species (Hack, 1982; Gallen et al., 2013).

It is possible that *Castaneides* previously had a more easterly Piedmont distribution which subsequently was lost as forests retreated to protected upland gorges after the Miocene optimum ~15 MYA (Kürschner et al., 2008). This hypothesis could explain why these salamander and spider populations became relictualized in the outlying Hickory Nut Gorge, surrounded as they are to the west by a much more mountainous terrain housing widespread congeners but which became extensively fragmented in the southern uplands. This scenario predicts the existence of another forested refuge on the southern Cumberland Plateau, and it was from here that the remainder of the *Castaneides* were subsequently founded as forest cover waxed and waned since the late Miocene. Martin et al.'s (2016) ancestral area analysis also hinted that the ancestor of *Castaneides* might have been a more widespread lowland and potentially arboreal form.

Karyotypic variation.—*Aneides* differs from other plethodontid genera in the extent of chromosomal variation (Sessions and Kezer, 1987). With few exceptions, plethodontids have 13 or 14 pairs of biarmed chromosomes and closely resemble each other. While all species of *Aneides* have 14 pairs of chromosomes, at least eight distinct karyotypes are found, three of them in the *A. aeneus* complex. One of these is "Morescalchi's *aeneus* III" (Sessions and Kezer, 1987), potentially important taxonomically but unavailable because of the lack of detailed geographic information (obtained by long-deceased Morescalchi from an unnamed animal dealer and suspected of being from the vicinity of Cumberland County, Tennessee). Morescalchi's materials, stored in Italy, were destroyed in the Naples earthquake in 1980. This region lies between sites where "*aeneus* I" (to the north) and "*aeneus* II" (to the southwest) have been recorded. In their map (fig. 13), Sessions and Kezer (1987) show *aeneus* I and II in sympatry (p. 24: "at one locality, near Chattanooga, Tennessee, a single specimen with the *aeneus* I karyotype has been detected among mainly aeneus II individuals"). A specimen from Hickory Nut Gorge has the *aeneus* I karyotype.

The report of Sessions and Kezer (1987) suggests that three karyotypes are found in eastern Tennessee, two of them in sympatry. In a recent communication to DBW (29 May 2018), Sessions provided additional details and corrected errors. For the published study, he examined three specimens of *A. aeneus* from "near Chattanooga", which he now specifies as from 4 miles S Sherwood at Buck Creek Cove, Franklin Co., Tennessee (collected by Wayne Van Devender). This site is just a few km west of Nickajack Cave (now mainly inundated by a reservoir), the type locality of *A. aeneus*. These specimens were sent to Kezer in Eugene, Oregon. Sessions took one to Berkeley, California, for his studies, and Kezer examined the other two. In a letter to Sessions (dated 29 November 1981), Kezer reports that the specimens he examined had completely bi-armed chromosomes (i.e., *aeneus* II). Sessions double-checked his lab notes from the time and finds that his single specimen also was *aeneus* II. He cannot explain how an error was made in the published version, but he concludes: "there was no evidence of co-occurrence of *aeneus* I and II at that site". Sessions sent DBW a complete list of specimens examined. There were no other specimens from Tennessee, and only the single specimen from Hickory Nut Gorge from North Carolina. They did have specimens from two sites in Alabama, both *aeneus* II. The map in their publication (fig. 13) is accordingly flawed and must be used with extreme care. Importantly for our current work, the specimen from Hickory Nut Gorge is *aeneus* I, which is found only at this site and from Kentucky and Virginia northwards to Maryland and Ohio, and nowhere else in any species of *Aneides* or any other plethodontid. The finding of the *aeneus* I karyotype in Hickory Nut Gorge is important in showing a sharp difference between this region and the region of the type locality of *A. aeneus*, very near the Tennessee–Alabama border.

A cladogram of chromosomal characters in *Aneides* shows the clade differing from the outgroup used (*Plethodon*) in two shared derived traits (Session and Kezer, 1987: fig. 15). *Aneides aeneus* differs further from other members of the genus in two more traits, and *aeneus* I and II are sister taxa. Karyotypic differences reported by Sessions and Kezer in the *A. ferreus* complex later proved to be species specific: *ferreus* I = *Aneides ferreus*, and *ferreus* II = *Aneides vagrans* (Jackman, 1998).

Conservation implications.—*Aneides caryaensis* faces pressing conservation concerns and need for management. This species has an extremely narrow range and is reported from fewer than 25 localities, despite extensive searches. The status of these populations is unknown, yet based on the personal observations of several of the authors, densities appear to be very low at all of the known localities, including those that had higher surveyed (by the North Carolina Wildlife Resource Commission) densities, often with only one to three individuals observed during a site visit. Additionally, these field observations are supported by our estimates of genetic diversity (Table 3), which indicate that populations of *A. caryaensis* are experiencing a high amount of inbreeding, a clear signature of small population sizes (Frankham et al., 2010). High levels of inbreeding can have detrimental effects on populations, including a decrease in fecundity that can in turn lead to lower population sizes and increased inbreeding, a feedback cycle often referred to as "the extinction vortex" (Frankham et al., 2010). Habitat loss and fragmentation resulting from the rapid rate of tourism, real estate development, and transportation and energy infrastructure within the Hickory Nut Gorge is likely the largest direct threat to the long-term survival of *A. caryaensis*. Habitat fragmentation and loss can easily occur for this species, as habitat features preferred by *A. caryaensis* tend to be distributed in patches across the landscape and are susceptible to disturbance from anthropogenic activities.

Similar to *A. caryaensis*, the BRE populations of *A. aeneus* also face a myriad of threats and conservation concerns. Although some populations in the BRE appeared to be recovering from the dramatic (98%) declines of the 1980s and 90s (Corser, 2001), they have been experiencing declines since at least 2005 (Apodaca and Williams, unpubl. data). The low present-day diversity observed for the BRE populations included in our study (Table 3) seems to reflect these declines. Importantly, the subpopulation structure within the BRE and apparent admixture between subpopulations attests to this complex demographic history. Although we have not explicitly tested demographic hypotheses for the BRE populations, our results imply either 1) secondary contact among previously isolated populations or 2) ongoing divergence. Future work studying the BRE populations would benefit from a thorough investigation of their demographic history.

Regardless, the low present day diversity of these populations in conjunction with their historical and continued declines emphasizes a great need to conserve and manage current populations and to develop novel approaches to mitigate threats. Necessary conservation actions will involve 1) protecting areas of known and potential habitat from certain types of logging, development, and collecting, and 2) maintaining a permeable matrix of habitat between such areas to facilitate dispersal.

In accordance with the mitochondrial and nuclear distinctiveness of this lineage, we propose the recognition of the BRE lineage of the *A. aeneus* complex as an ESU. This proposal is compatible with the criterion for recognizing ESUs outlined by Moritz (1994). In his article, Moritz (1994) defined an ESU as being "reciprocally monophyletic for mtDNA alleles and show significant divergence of alleles at nuclear loci." We have demonstrated this for the BRE lineage of *A. aeneus* despite apparent population structuring. Although our OTU sampling for the SNP dataset is far less than that of our mitochondrial dataset, we have recovered reciprocal monophyly for the BRE populations. Further, the BRE lineage, as identified herein, is compatible with a number of other definitions of ESUs as outlined by Funk et al. (2012; e.g., Avise, 1994; Vogler and DeSalle, 1994; Fraser and Bernatchez, 2001). As such, we anticipate our recommendation for the recognition of the BRE populations of *A.*

aeneus as an ESU to be uncontentious. Recognition of the BRE lineage as an ESU will facilitate its conservation, as ESUs are granted legal protection under the Endangered Species Act. We do note, however, that species delimitation methods included BRE populations with northern Appalachian lineages (Fig. 3). Interestingly, the PTP designated the southern Appalachian lineage as a distinct species. This is coincident with long branch-lengths as estimated by RAxML. Too little is known about these populations at present to designate this lineage as an ESU, but we anticipate that additional investigation of this lineage will uncover previously unrecognized differentiation.

Conclusions.—Herein, we have used multiple lines of evidence to identify four lineages within *Castaneides*. The newly described North Carolina endemic, *A. caryaensis,* is restricted to an extremely narrow geographic range and is in need of immediate protection. However, little is known about the ecology of this species, as most work done on *Castaneides* has been conducted on more westerly populations and the Blue Ridge Escarpment lineage (e.g., Gordon, 1952; Snyder, 1983, 1991; Cupp, 1991; Corser, 2001; Waldron and Humphries, 2005; Smith et al., 2017). Perhaps the most important research needs at present are population surveys, habitat management guidelines, landscape genetic analyses, and habitat use studies. We emphasize, however, that such research needs are not restricted to *A. caryaensis*. The BRE ESU of *A. aeneus* is also of great conservation concern and faces similar threats as *A. caryaensis*. In summary, we hope that in describing *A. caryaensis*, more work will be conducted to more rigorously describe the cryptic diversity harbored within *Castaneides*.

DATA ACCESSIBILITY

Supplemental information is available at https://www.copeiajournal.org/ch-18-052.

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EDITORIAL NOTES AND NEWS

NOTE FROM THE EDITOR

Copeia is happy to announce that its new platform for manuscript submission and review, PeerTrack, is now active at:

https://editorialmanager.com/copeia

One of the benefits of our new manuscript tracking system is that *Copeia* will have double blind review in which authors may choose to be anonymous to reviewers. To make this possible, authors will need to upload the title page and acknowledgments of their submission separately from the manuscript file.

If you were already registered in the old system (AllenTrack) as an author or reviewer, you should have received an email in early January announcing the new system. This email included a username and a link to set your password. If you wish to change your username, please log in, then click "Update My Information" in the blue menu bar. If you need any assistance with the new system, please contact technical support (support@peertrack.net).

If you submitted a manuscript to AllenTrack prior to 31 December 2019, it will remain in that system through the end of 2020, and all revisions and reviews will be handled there.

If you have any questions or concerns, please feel free to contact the Editor (copeiaeditor@gmail.com) or Production Editor (copeiajournal@gmail.com).

Thank you for your continued support of *Copeia*,
Leo Smith
Editor, *Copeia*

GILBERT ICHTHYOLOGICAL SOCIETY

THE Gilbert Ichthyological Society (GIS) held its 31st annual meeting 20–22 September 2019 at Pack Forest in Eatonville, Washington. The GIS is named for celebrated ichthyologist Charles Henry Gilbert (1859–1928), who either by himself or as coauthor (most often with David Starr Jordan) was responsible for the discovery and naming of approximately 117 new genera and about 620 new species of fishes, including about 25% of the fish fauna of Washington and Oregon. The society was resurrected in 1989 from the Gilbert Fisheries Society, a short-lived organization founded in 1931 at the then Department of Fisheries, University of Washington (see *Copeia* 1931: 71). The primary purpose of the GIS is to foster communication in the Pacific Northwest concerning all things ichthyological.

The 2019 meeting was attended by 33 ichthyologists from Washington, Oregon, and California. Christina Murphy began her term as the 2020 President of the society. Anne Marshall was elected President for 2021. Ten new Fellows were inducted into the society, bringing the total membership to 338 fellows. Talks were presented by C. A. Murphy, M. Gomez-Buckley, R. Buckley, M. Busby, M. Moser, R. J. Arnold, T. Desvignes, J. Healy, T. Buser, S. T. Friedman, A. Bronson, K. C. Hall, K. E. Cohen, S. Ghods, T. Clardy, J. Egge, J. M. Huie, D. Stevenson, L. Tornabene, C. Atta, J. Orr, J. Gardner, E. McFarland, and B. W. Frable. The banquet keynote address was delivered by Karen Crow, with the title "How the devil ray got its horns: the genetic basis of body plan remodeling in manta rays and their relatives." A full presentation list is available online at the GIS website, at http://www.gilbertsociety.org/.

Next year's 32nd annual meeting of the GIS will be held 2–4 October 2020 at Government Camp, Mt. Hood, Oregon. For more information about the society, visit the GIS website or contact Brian Sidlauskas, GIS Secretary, Oregon State University Department of Fisheries and Wildlife, Corvallis, Oregon 97331; email: Brian.Sidlauskas@oregonstate.edu.

CORRIGENDUM

CORRIGENDUM to: "A New Green Salamander in the Southern Appalachians: Evolutionary History of *Aneides aeneus* and Implications for Management and Conservation with the Description of a Cryptic Microendemic Species" by Austin Patton, Joseph J. Apodaca, Jeffrey D. Corser, Christopher R. Wilson, Lori A. Williams, Alan D. Cameron, and David B. Wake, published in *Copeia* 107(4), pages 748–763 (DOI: 10.1643/CH-18-052). On page 755, the holotype of *Aneides caryaensis* (NCSM 33389) is also listed in error as a paratype. The correct numbers for the paratype series are NCSM 33390–33391. We regret the error.

DOI: 10.1643/CT2020022

Attachment 17

Arboreal Habitat Use by the Green Salamander, *Aneides aeneus*, in South Carolina

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Arboreal Habitat Use by the Green Salamander, *Aneides aeneus*, in South Carolina

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ABSTRACT.—**Green Salamander (*Aneides aeneus*) habitat has been described traditionally as rock outcrop formations that contain moist, but not wet, crevices. Early studies of Green Salamander natural history claimed arboreal habitat was used secondarily to rock outcrops and in situations where more suitable habitat was unavailable. Although arboreal behavior of western *Aneides* has been well established, arboreal habitat has been deemed "not typical" for Green Salamanders. This study examined the extent to which Green Salamanders use arboreal habitat. Surveys were conducted between August 2001 and July 2004 at a study area in Pickens County, South Carolina. Salamander size influenced arboreal habitat use, but gender and reproductive condition did not. There was a positive relationship between tree diameter at breast height (DBH) and Green Salamander observations and a negative relationship between tree distance to rock outcrop and salamander observations. Tree selection did not reflect tree species relative dominance, and salamanders favored hardwoods over conifers. Seasonal use of arboreal habitat was distinct, implying that salamanders overwinter in rock outcrops and move into trees and logs at the onset of spring. Salamanders observed during summer were primarily arboreal, but they returned to rock outcrops in late fall. Researchers have largely overlooked arboreal habitat use by Green Salamanders, and consequently, the importance of arboreal habitat near rock outcrops has been underestimated. Arboreal habitat appears to be an important component of the life history of this declining species, and future survey and monitoring efforts should include searches of arboreal habitat.**

Given its unique habitat requirements and natural history, the Green Salamander (*Aneides aeneus*) is listed as a "species at risk" by the U. S. Fish and Wildlife Service. It is listed as critically imperiled, imperiled, or vulnerable in 10 of the 13 states in which it occurs. Interest in conservation of Green Salamanders piqued following a publication by Corser (2001), which reported a 98% decline of some populations within the Blue Ridge Escarpment since 1970. Speculation about why these populations declined has centered on synergistic effects of overcollection by researchers, fungal pathogens, climate change, and habitat loss (Corser, 2001). It is the latter component of such speculation that is of interest to this study.

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Green Salamander habitat has been described traditionally as limestone (Walker and Goodpaster, 1941), sandstone, granite, and schist formations (Netting and Richmond, 1932; Gordon and Smith, 1949; Schwartz, 1954; Bruce, 1968; Mount, 1975) that contain crevices that are moist but not wet. Petranka (1998) suggested that Green Salamanders have a patchy distribution caused by their dependence on rock outcrops. However, morphological and ecological traits of members of the genus *Aneides* suggest an arboreal lifestyle (Wake, 1963, 1966). Arboreal behavior by western *Aneides* has been well documented (Ritter, 1903; Fitch, 1936; Stebbins, 1951; Stelmock and Harestad, 1979; Whitaker et al., 1986; Davis, 2002), and there are numerous reports of Green Salamanders in arboreal or woody habitat (Brimley, 1927; Bishop, 1928; Pope, 1928; Welter and Barbour, 1940; Brimley, 1941; Fowler, 1947; Gordon and Smith, 1949; Gordon, 1952; Schwartz, 1954; Canterbury, 1991). Barbour (1971) described an arboreal observation in Kentucky, stating "They [Green Salamanders] sometimes reach a high population in logged-over areas where dead tree tops were left. They reached tremendous populations in the 1930s under the bark of the millions of dead chestnut trees in eastern Kentucky."

Other scientific literature suggested that Green Salamanders are not closely associated with arboreal habitat, and they are described as being "weakly" arboreal (Bishop, 1928). In the most extensive summary of Green Salamander life history, Gordon (1952) stated that Green Salamanders were only occasionally arboreal in the Blue Ridge Escarpment, and there has been little evidence to suggest that arboreal habitat is essential to healthy population structure for the species. Bruce (1968) suggested that arboreal habitat use is restricted to mixed-mesophytic forests of the Appalachian Plateau Province, where arboreal observations have been noted (Barbour, 1971). Currently, arboreal habitat is deemed "not typical" over most of the range of the species (Snyder, 1991).

Historically, search efforts for Green Salamanders focused on rock outcrops, although arboreal surveys occasionally were conducted. In such surveys, researchers focused on dead or dying trees (standing or fallen) with loose bark, rather than on live trees, and few studies yielded arboreal observations. Consequently, arboreal habitat was deemed secondary to rock outcrops as preferred habitat and in situations where more suitable habitat was not available (Gordon and Smith, 1949; Gordon, 1952; Woods, 1968; Barbour, 1971; Mount, 1975). Rock outcrops are still thought of as harboring the majority of salamanders within a given population. Evidence of reproduction or nesting in standing arboreal habitat is lacking, and there are few records of Green Salamanders nesting in logs.

In August 2001, we discovered nine Green Salamanders on a single American Beech (*Fagus grandifolia*) at a South Carolina study site and observed 14 individuals on 14 different trees during a subsequent survey of the area. Thus, we began a study on the arboreal habits of Green Salamanders. Specifically, we addressed four questions. (1) How does habitat use (arboreal vs. rock outcrop) vary by size-class and gender of salamanders? (2) Do Green Salamanders favor specific tree species over others? (3) Does tree diameter and distance to rock outcrop influence arboreal habitat use? (4) Does arboreal habitat use vary seasonally?

Materials and Methods

Study Area.—The study area was surveyed between August 2001 and July 2004. The exact location of the study area is not reported because of a history of overcollection leading to population declines at sites reported in previously published papers. The study area was located at the base of the Blue Ridge Escarpment (elevation = 300 m) in Pickens County, South Carolina. Mixed pine-hardwood forest dominated the lower portion of the study site, and more xeric species (e.g., Virginia Pine) dominated the area above the main outcrop. Twelve species of trees (> 5 cm Diameter at Breast Height [DBH]) were documented within the study area. Rhododendron was numerous, although it had a low relative dominance because of the size of its stems. Cores extracted from a Red Oak (*Quercus ruber*), Tulip Poplar (*Lirodendroan tulipifera*), and Eastern Hemlock (*Tsuga canadensis*) indicated the stand was uneven-aged, with larger trees ranging from 85–100 yr. Several granite rock outcrops exposed along the floodplain of a medium-sized stream characterized the site. The main rock outcrop was approximately 80 m long and 10 m high, with numerous crevices and fissures. The stream created a boundary that limited searches to approximately 20 m from the main rock outcrop. The area between the main rock outcrop and the stream was approximately 25 × 72 m (0.18 ha).

Surveys.—We attempted to conduct surveys during optimal weather conditions (i.e., overcast and/or drizzling), but time constraints and logistical problems often meant that the study area was surveyed during what would be considered suboptimal conditions (i.e., sunny and dry). Surveys were timed and involved searching all available habitat within the study area, including the ground, trees, logs, and rock outcrops. Flashlights were used during both day and night searches to scan crevices and faces of emergent rock outcrops, trees, and logs. Even during daytime searches, light from the flashlights reflected off of the skin of salamanders and made them easier to observe on trees. Inaccessible individuals were coaxed from their crevice using a wire or stick with a hook and blunt tip in order to measure age classes.

Tree searches were conducted from the ground, although it was difficult to observe salamanders above approximately 10 m; salamanders could sometimes be seen easily with binoculars. Ground searches involved searching the ground surface for active salamanders, but leaf litter searches (sifting through leaf litter and turning of logs) were not conducted. When a salamander was observed, we recorded whether it was within a crevice or active on the substrate surface. Captured individuals were placed in a plastic bag, sexed, and measured for snout–vent length (SVL), total length (TL), and the salamander was released at the exact capture location. The dorsal pattern of each salamander was photographed for individual recognition as part of a concurrent mark-recapture and movement study. Individual identification allowed for observations of the number of individual salamanders found on trees. However, not all individuals counted during surveys were accessible for capture and individual recognition. Therefore, unless specified, data are presented as the number of salamander observations.

Salamanders were placed into one of three age classes according to SVL, including adults (> 44.5 mm), subadults (29.5–44.4 mm), and juveniles (< 29.4 mm). Sex was determined based on the presence of mental glands in males and eggs in gravid females. When neither mental glands nor eggs were observed in individuals greater than 44.5 mm SVL during the breeding season, we assumed they were nongravid females. Sex could not be determined when dimorphic characters were not present outside of the breeding season. Measurements taken at each capture location included substrate type (rock, tree, or log), height, tree or log diameter, and distance to nearest rock outcrop. Tree Diameter was measured at breast height, and log diameter was measured at the center of each log. The "distance to nearest rock outcrop" measurements were made to one particular rock where salamanders appeared to overwinter (unpubl. data).

Data Analysis.—All statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC, 2002). Negative binomial Poisson regression (PROC GENMOD) was used to examine the relationship between the number of salamander observations, tree size (DBH), and distance to the nearest rock outcrop. Because salamanders had different levels of detectability on logs and trees, observations on logs were excluded from the Poisson regression analysis. However, observations made on logs and trees were clumped for most other analyses. One-way frequency tables were used to examine the distribution of salamanders on rocks and arboreal habitat (trees and logs) throughout the year. Chi-square analysis was used to examine differences in size class, gender, and reproductive condition with respect to arboreal habitat use. Only active season (April through October) observations were used for descriptions of tree and log use patterns. Because salamanders were only observed in rocks during the winter, we assumed they were overwintering in rock outcrops. Therefore, we classified the active season as between April and October, when salamanders were found on rocks, trees, and logs. Seasonal activity patterns were described and referenced to the number of adjusted person hours spent searching for salamanders. We assumed that it took at least five minutes to process individuals based on the length of time required to handle salamanders during early surveys. Therefore, person hours were adjusted so that five minutes were subtracted for each salamander captured from the total number of minutes spent searching the study area.

RESULTS

We recorded 491 Green Salamander observations within the study area during 71 surveys. Of 345 salamander observations during the active season, 143 were found on rocks (113 individuals), 150 on trees (71 individuals), and 52 on logs (11 individuals).

Habitat Use by Size-Class and Sex.—More adults (N = 228) were observed than subadults (N = 108) and juveniles (N = 115) during this study. Use of rocks, trees, and logs differed among salamander size classes ($\chi^2 = 20.61$, df = 4, $P < 0.001$). Juveniles were observed most often on trees, whereas adults and subadults were more frequently observed on rocks (Fig. 1A). Trees, logs, and rock outcrops were used in proportion to the number of males (N = 54) and females (N = 41) observed ($\chi^2 = 4.50$, df = 2, $P > 0.05$, Fig. 1B). Both gravid (N = 16) and nongravid (N = 21) females were observed on rocks, trees, and logs (Fig. 1B), although low sample size on logs only allowed for comparisons between rock outcrops and trees. Trees and rock outcrops were used in proportion to the number of gravid and nongravid females observed ($\chi^2 = 2.89$, df = 1, $P > 0.05$, Fig. 1B). Evidence of arboreal nesting was observed on one occasion in October 2003, when hatchlings were seen descending a Black Walnut (*Juglans nigra*) during a fall survey.

Tree Selection: Relative Dominance, Size, and Distance from Rock Outcrop.—Green Salamanders were observed on 35 of the 63 trees regularly

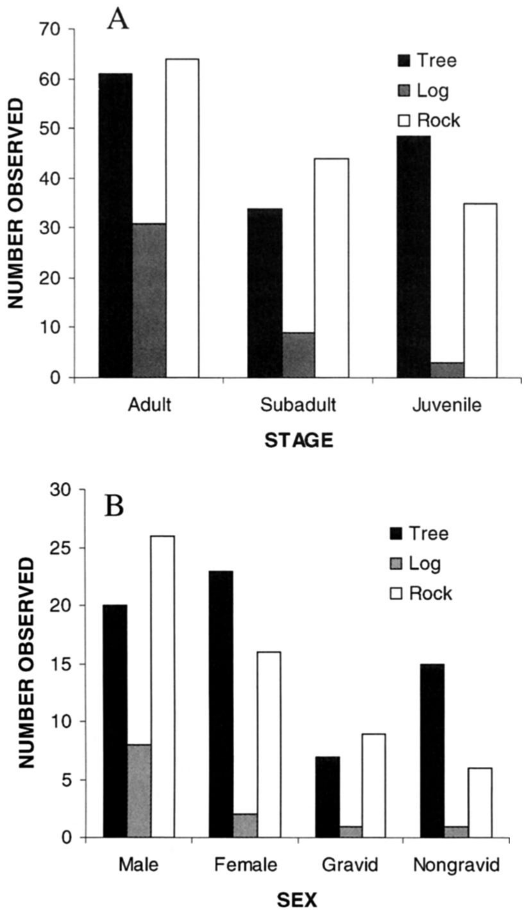

FIG. 1. (A) Number of observations of Green Salamanders in rock outcrops, trees, and logs during the active season (April through October). (B) Number of observations of male and female (gravid and nongravid) Green Salamanders on trees, logs, and rock outcrops during the active season within the study area.

surveyed during the study, including all 12 of the tree species available within the area. Only one individual was found beneath the bark of a dead tree; all other arboreal observations, with the exception of those on logs, occurred on living trees. Observations of arboreal salamanders were not proportional to the relative dominance of tree species ($\chi^2 = 234.26$, df = 11, $P < 0.0001$, Fig. 2). Although two of the three most dominant tree species were conifers (Fig. 2), Green Salamanders were observed more often on hardwood trees ($N = 137$) than coniferous trees ($N = 13$), and salamanders used hardwoods disproportionally to their abundance ($\chi^2 = 102.51$, df = 1, $P < 0.0001$). Multiple salamanders were sometimes found together on the same tree. For instance, as many as nine individuals were observed on a single American Beech at the same time.

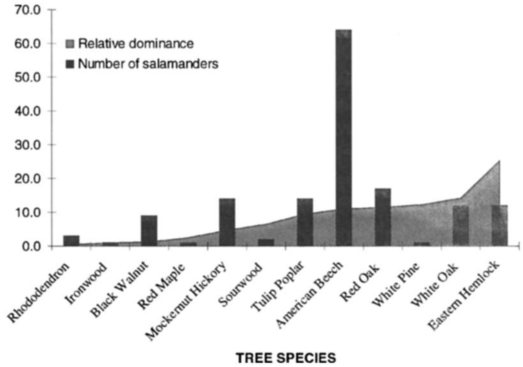

FIG. 2. Number of observations of Green Salamanders in tree species that occurred within the study area. Numbers are presented with respect to the relative dominance of each tree species within the study area.

On average, salamanders were observed 1.3 m high ($N = 182$, SD = 0.61, range = 0.1–3.6) on rocks, and 2.3 m high ($N = 137$, SD = 2.67, range = 0.25–21) on trees. The number of observations of adults, subadults, and juveniles was proportional on rocks and trees ($\chi^2 = 5.29$, df = 2, $P > 0.05$), and they were combined in the negative binomial Poisson regression model. The original Poisson model included the y-intercept and predictor variables DBH and distance to rock outcrop, but the y-intercept was not significant (estimate = –0.1975, SE = 0.43, $\chi^2 = 0.21$, $P > 0.05$), and there was a problem with overdispersion (Pearson $\chi^2 = 89.38$). Therefore, we used a negative binomial linear model to predict the number of Green Salamander observations in trees with respect to DBH and distance, without the inclusion of the y-intercept. The model indicated a positive relationship between DBH (estimate = 0.1203, SE = 0.01, $\chi^2 = 73.56$, $P < 0.0001$) and salamander observations (Pearson $\chi^2 = 32.40$), and a negative relationship between distance to rock outcrop and the number of salamander observations (estimate = –0.0744, SE = 0.03, $\chi^2 = 6.02$, $P = 0.01$).

Seasonal Habitat Use.—Strong seasonal activity patterns on rock outcrop and arboreal habitat were evident without regard to sampling effort (Fig. 3). Seasonal shifts in habitat use suggest salamanders overwinter in rock outcrops and move into woody or arboreal habitat beginning in March, where they appear to remain throughout the breeding/nesting season before returning to rock outcrops in October and November (Fig. 3). Fluctuations in activity on rock outcrops were pronounced ($\chi^2 = 21.39$, df = 10, $P < 0.05$), because few individuals were observed on the rocks during summer months (Fig. 3). However, when arboreal and rock outcrop observations were combined for analysis, salamander obser-

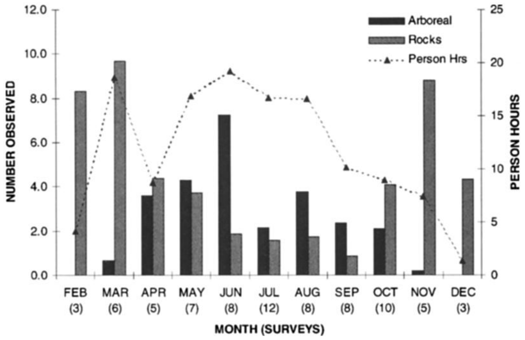

FIG. 3. Seasonal use of arboreal and rock outcrop habitat by Green Salamanders. Numbers in parentheses below each month refer to the number of surveys conducted during the respective month between August 2001 and July 2004. Number observed refers to the number of Green Salamanders observed per survey, per month.

vations remained constant throughout the year ($\chi^2 = 8.26$, df = 10, $P > 0.05$).

DISCUSSION

Sampling bias toward rock outcrops and dead trees probably caused researchers to overlook Green Salamanders in arboreal habitat during the active season in previous studies. Until now, scientific literature has been devoid of information suggesting trees (live or dead) and logs play a major role in the behavior and population ecology of Green Salamanders. Unlike early reports of Green Salamander habitat use, where arboreal searches were uncommon and focused on dead or dying trees, we suggest that dead trees are not necessary for Green Salamander activity in arboreal habitat. To the contrary, only one Green Salamander was observed on a dead tree during this study. The presence of Green Salamanders of all size classes and both sexes (regardless of reproductive condition) on trees and logs throughout the active season, along with evidence of nesting in trees, clearly suggests that arboreal structure is at least as important as rock outcrops to the natural history of Green Salamanders.

Habitat Use by Size-Class and Sex.—The frequency at which adults, subadults and juveniles were observed did not necessarily reflect population demographics. A plausible explanation for the observed bias toward adults is that smaller salamanders were more difficult to detect. Therefore, the frequency of size class observations is likely the result of sampling bias.

Our observations suggest gravid females select both arboreal and rock outcrop habitats for breeding and nesting. Gravid females were found on trees and logs throughout the breeding season and just prior to the onset of nesting season (mid-July). Although no females were observed with nests in trees, it seems unlikely that gravid females would be far from desired nesting habitat just prior to depositing eggs. In addition, sexually mature males with mental glands were observed in high numbers on trees throughout the breeding season, which suggests that they were defending nesting habitat (Cupp, 1980) and attempting to breed with females that were in trees.

Only three nests (all in rock crevices) were discovered during the study period, but two failed before hatching. Nevertheless, hatchlings were abundant at the site. The observation of a cluster of hatchlings in a tree in October infers that the individuals were dispersing from a nest within the tree. The tree on which the hatchlings were observed had numerous tree holes that appeared suitable for nesting. Whether nesting in trees is common, preferred, or related to a lack of suitable nesting crevices in rock outcrops is unknown.

Tree Selection: Relative Dominance, Size, and Distance from Rock Outcrop.—The observation that Green Salamanders did not use tree species based on tree relative dominance and that salamanders were disproportionately found on hardwoods, indicated that salamanders were selecting hardwood tree species. Although not quantified, the major similarity among hardwood trees selected by Green Salamanders was that they were large trees with holes of various shapes and sizes on the main stem. Regression analysis further elucidated why Green Salamanders selected specific trees. The effect of tree size and distance from rock outcrops offered insight into why 58 Green Salamander observations were made on a single American beech. This tree had a 75 cm DBH, and was located within 2 m of the rock outcrop.

Trees used by Green Salamanders appeared to provide cover through a series of bark characteristics. During dry days, individuals were often seen under flaps of bark on various tree species. Red Oak, Tulip Poplar, and White Oak have bark that tends to become flaky and furrowed enough for an adult salamander to hide beneath as the tree becomes older and larger. Green Salamanders were well camouflaged on older American Beech trees, despite their smooth bark, because the bark was often lichen-covered. Further, large beeches are oftentimes hollow and have numerous holes formed by fallen branches. One adult Green Salamander was observed halfway out of a tree hole in an American Beech at a height of 21 m (viewed with binoculars). These large, lichen-covered trees with numerous tree holes appeared to provide extensive habitat for Green Salamanders.

Seasonal Habitat Use.—The extent to which Green Salamanders disperse from rock outcrops is poorly understood. Researchers have suggested that Green Salamanders are likely to disperse from rock outcrops when suitable crevices for breeding and nesting are not available (Gordon, 1952; Woods, 1968). This implies that salamanders overwintering in rock outcrops that lack suitable breeding crevices have to travel to find nesting habitat. Green Salamanders did not appear to disperse from rock outcrops solely for breeding and nesting opportunities. Rather, individuals began dispersing from rock outcrops as soon as environmental conditions were suitable in the spring. Breeding was clearly not the only reason salamanders used trees, as evidenced by observations of juveniles and subadults in arboreal habitat throughout the active season.

Seasonal use of arboreal habitat was distinct. Green Salamanders migrated from their winter refugia in the rock outcrop to trees and logs as soon as the threat of freezing weather ceased (late March through early April), and individuals returned to the rock outcrops as colder weather approached (late October through early November). Green Salamanders have been historically difficult to detect during summer months, whereas spring and fall surveys have been more successful at detecting salamanders. Because the number of observations was low during summer months, researchers have speculated that salamanders retreated into crevices to avoid hot summer conditions and that salamander activity decreased during summer months. Specifically, Gordon (1952) reported an annual cycle of Green Salamander activity in the Blue Ridge Escarpment that included a "pre-hibernation dispersal and aggregation period" (late April through May) and a fall "post-hibernation aggregation and dispersal period" (late September through November). These aggregations consisted of numerous adult salamanders and hatchlings that were thought to be gathering on the rock outcrop just prior to and just after winter months; other studies have reported similar results (Woods, 1968; Cupp, 1991). Our study showed a similar trend with regard to salamander observations on rock outcrops. However, when arboreal habitat was included in analysis, the number of Green Salamander observations was not significantly different among months. Our results suggest that spring and fall peaks of salamander observations on rock outcrops do not reflect seasonal salamander activity as outlined by Gordon (1952). Rather, there appears to be a seasonal shift in habitat use. This habitat shift is only evident when arboreal habitats are surveyed. Although not significant, the number of salamander observations decreased in July, even though search hours remained high. Given the seasonal shift toward arboreal habitat, we believe the lower number of observations in July was caused by salamanders climbing into the tree canopy, where they were not detectible.

Conservation Implications.—Researchers have largely overlooked Green Salamander arboreal habitat use, and consequently, the importance of arboreal habitat near rock outcrops has been underestimated. Because tree canopies were not searched during the study, and because of difficulty in observing salamanders above 10 m, our results present an underestimation of the importance of arboreal habitat to Green Salamanders. It is clear that Green Salamanders can be highly arboreal, and we present conservative estimates of the importance of, and the extent to which, Green Salamanders use arboreal habitat. In addition to our study area in South Carolina, we have observed numerous Green Salamanders in arboreal habitat in the Allegheny Mountains, Appalachian Plateau, and Blue Ridge Provinces within the states of West Virginia, Ohio, Georgia, and other areas of South Carolina (unpubl. data).

Forest management around rock outcrops may directly affect movements and habitat use by Green Salamanders. We believe sampling bias toward rock outcrops has led to inaccurate descriptions of Green Salamander habitat requirements. Consequently, managers have had no indication that leaving trees around rock outcrops may prove important for this sensitive species, aside from providing shade for outcrops. Future research efforts should examine the benefits of leaving tree buffers around rock outcrops in managed landscapes, and determine how far Green Salamanders disperse within an active season. Our most distant Green Salamander observation was 42 m from the nearest rock outcrop at a study site in Georgia (Unpubl. data). Efforts to survey and monitor Green Salamander populations should incorporate arboreal habitat, including canopy searches. Tree climbing will greatly benefit arboreal searches and increase the probability of encountering nests in trees. Finally, certain species of large diameter trees appear to be favored by Green Salamanders, and these issues should be considered in forest planning within the range of this unique salamander.

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Attachment 18

JJ Apodaca comments on the Forest Service green salamander (*Aneides aeneus*) surveys for the "Southside Project"

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August 27th, 2018

Comments on the Forest Service green salamander (*Aneides aeneus)* surveys for the "Southside Project"

In general, I commend the Forest Service (FS) for considering the presence of *Aneides aeneus* in silvicultural practices in the Nantahala NF, and for removing stands 29-17 and 41-39 in response to the presence of green salamanders. However, as a species of conservation concern, it also appears that the FS was remiss in their efforts to thoroughly survey for the species. Green salamanders are an extremely difficult species to detect due to the fact that their presence in rock crevices (where surveys almost exclusively occur) is very ephemeral in nature. In fact, recent research employing Bayesian models for the species have all pointed to different factors having the most influence on detectability (time of day; Newman 2017, experience of surveyor and outcrop features; John 2017, and season; Novarro et al. in prep). Thus, it is clear that in order to have any certainty regarding the presence of the species, one must design a survey protocol that minimally includes qualified (and experienced) surveyors and multiple surveys that cover various times of year, environmental factors (humidity, temp, etc.), and time of day that an individual outcrop is surveyed. Further, in order to have a high level of confidence, a survey should include estimates of survey effort (e.g. amount of time surveyed per meter of rock outcrop) and attempts should be made to identify all possible rock outcrops (an extremely difficult task during the growing season due to the density of many of these habitats (unless the use of remote sensing with temperature capability is employed).

Efforts to survey for *A. aeneus* for this project included only one of the above factors. While Charles Lawson (formerly of the NCWRC) and Mike Osborne are experienced and qualified for such a task, it appears as though the FS's investigation with these skilled surveyors was limited, involving only one survey from Osborne and two with Lawson, and at that, mostly limited to known localities (though at least one new locality was recorded). We have no record of which stands were surveyed for how long and whether these surveys were exhaustive or selective. Research suggests that detection rate of known localities is roughly between 20 and 31 percent when surveys are conducted by an experienced surveyor (John 2017). The detection of a green salamander at a novel (previously unknown site) is roughly 10% (Newman 2017). Thus, even with two qualified surveys, the probability of detecting all localities within the proposed stands is extremely low.

Our knowledge of green salamander biology has grown immensely in the last 2 decades, elucidating parts of their life history that we knew little about. For example, we now know that they spend a good amount of their life cycle in the canopy and not just in rock crevices (Wilson 2003, Waldron & Humphries 2005), a trait commonly found in other members of the genus *Aneides*. However, we still know relatively nothing about their dispersal (distance, timing, rate, etc.). Strong genetic evidence suggests that many (if not most) populations of green salamanders in North Carolina are isolated and in need of habitat connectivity (Apodaca 2015). Simply buffering existing rocks rather than leaving larger pockets of suitable habitat will only lead to the degradation of metapopulation connectivity and will thus lead to the decline of genetic diversity, lower recruitment, increased allee effects, and eventually lead to the loss of populations.

It should also be noted that populations within the Nantahala NF have declined precipitously at least 3 times in the last 4 decades, the Mid 70s, mid 90s, and late 2000s (Corser 2001 and unpublished data). Current evidence suggests that this has had a devastating effect on population survivability estimates and genetic diversity (Apodaca in prep). Thus, it is incredibly important to conserve any remaining populations and the existence of a functioning metapopulation. The loss of small undocumented populations can have a devastating effect on metapopulation function.

I base these comments on my background and qualifications as a biologist that focuses on southeastern amphibians and reptiles, including green salamanders and several other species of conservation concern. I received a B.S. in Biology at the University of South Florida in 2004, a Ph.D. in Biology from the University of Alabama in 2010 (focused on salamander conservation), and held a post-doctoral research position at Florida State University. I have been working in amphibian and reptile conservation and research for nearly two decades and I was the national co-chair for Partners in Amphibian and Reptile Conservation (PARC), the largest amphibian and reptile conservation organization in the world, for four years and am currently the Director of Science and Associate Executive Director for Amphibian and Reptile Conservancy (ARC) and the founder of Tangled Bank Conservation. My ongoing research combines several fields and methods (i.e. conservation genetics, habitat-modeling, life history studies, etc.) in order to develop and inform optimal conservation and management decisions.

J.J. Apodaca, PhD
Founder and Lead Scientist

Attachment 19

Regional Forester's Sensitive Species List – Southern Region

Regional Forester's Sensitive Species List

Revised : 02/15/2018

USDA Forest Service Southern Region

Group	Species	Forest														Total Occurences
		1	2	3	4	5	6	7	8	9	10	11	12	13	60	
		Alabama	Daniel Boone	Chatt-Oconee	Cherokee	NFs Florida	Kisatchie	Mississppi	GW Jeff	Ouachita	Ozark	NFs North Carolina	Sumter	NF&G Texas	LBL	
Crustacean	Caecidotea incurva								●							1
Crustacean	Cambarus bouchardi		●													1
Crustacean	Cambarus causeyi										●					1
Crustacean	Cambarus chaugaensis											●	●			2
Crustacean	Cambarus conasaugaensi				●											1
Crustacean	Cambarus cymatilis			●												1
Crustacean	Cambarus extraneus			●												1
Crustacean	Cambarus georgiae			●								●				2
Crustacean	Cambarus parrishi			●								●				2
Crustacean	Cambarus speciosus			●												1
Crustacean	Cambarus veteranus								●							1
Crustacean	Crangonyx castellanum		●													1
Crustacean	Crangonyx grandimanus					●										1

Crustacean	Crangonyx hobbsi					●										1
Crustacean	Distocambarus crockeri												●			1
Crustacean	Distocambarus youngineri												●			1
Crustacean	Fallicambarus danielae							●								1
Crustacean	Fallicambarus gordoni							●								1
Crustacean	Fallicambarus wallsi													●		1
Crustacean	Miktoniscus racovitzai								●							1
Crustacean	Orconectes menae									●						1
Crustacean	Orconectes packardi		●													1
Crustacean	Orconectes saxatilis									●						1
Crustacean	Orconectes williamsi										●					1
Crustacean	Procambarus attiguus					●										1
Crustacean	Procambarus barbiger							●								1
Crustacean	Procambarus delicatus					●										1
Crustacean	Procambarus fitzpatricki							●								1
Crustacean	Procambarus horsti					●										1
Crustacean	Procambarus orcinus					●										1
Crustacean	Procambarus reimeri									●						1
Crustacean	Remasellus parvus					●										1
Crustacean	Stygobromus abditus								●							1
Crustacean	Stygobromus carolinensis											●				1
Crustacean	Stygobromus emarginatus								●							1
Crustacean	Stygobromus gracilipes								●							1
Crustacean	Stygobromus hoffmani								●							1
Crustacean	Stygobromus mundus								●							1
Crustacean	Stygobromus pollostus								●							1
Crustacean	Stygobromus spinatus								●							1
Fish	Acipenser fulvescens		●		●											2
Fish	Alosa alabamae	●				●		●								3
Fish	Ammocrypta clara								●					●		2
Fish	Crystallaria asprella	●														1
Fish	Cyprinella callitaenia			●		●										2
Fish	Erimystax insignis		●	●	●				●			●				5
Fish	Etheostoma acuticeps				●				●			●				3
Fish	Etheostoma bellator	●														1
Fish	Etheostoma brevirostrum	●		●	●											3
Fish	Etheostoma cinereum		●						●							2
Fish	Etheostoma ditrema	●		●												2

Fish	Etheostoma douglasi	●														1
Fish	Etheostoma maculatum		●													1
Fish	Etheostoma pallididorsum									●						1
Fish	Etheostoma raneyi							●								1
Fish	Etheostoma sagitta sagitta		●													1
Fish	Etheostoma tippecanoe								●							1
Fish	Etheostoma vulneratum			●	●				●			●				4
Fish	Hybopsis lineapunctata	●		●												2
Fish	Ichthyomyzon fossor		●													1
Fish	Ichthyomyzon greeleyi		●		●				●							3
Fish	Lythrurus snelsoni									●						1
Fish	Micropterus coosae				●							●	●			3
Fish	Micropterus notius					●										1
Fish	Moxostoma robustum			●									●			2
Fish	Moxostoma sp. 1			●		●										2
Fish	Notropis ariommus								●							1
Fish	Notropis bifrenatus								●			●				2
Fish	Notropis chalybaeus	●												●		2
Fish	Notropis ortenburgeri									●						1
Fish	Notropis ozarcanus										●					1
Fish	Notropis perpallidus									●						1
Fish	Notropis semperasper								●							1
Fish	Notropis suttkusi									●						1
Fish	Notropis uranoscopus	●														1
Fish	Noturus gilberti								●							1
Fish	Noturus gladiator							●								1
Fish	Noturus lachneri									●						1
Fish	Noturus munitus	●		●												2
Fish	Noturus stigmosus		●													1
Fish	Noturus taylori									●						1
Fish	Percina aurora							●								1
Fish	Percina brucethompsoni									●						1
Fish	Percina burtoni				●				●			●				3
Fish	Percina kusha			●	●											2
Fish	Percina macrocephala		●													1
Fish	Percina nasuta									●	●					2
Fish	Percina sipsi	●														1
Fish	Percina squamata		●	●	●							●				4

Fish	Percina uranidea									●						1
Fish	Percina williamsi								●							1
Fish	Phenacobius teretulus								●							1
Fish	Pteronotropis welaka					●		●								2
Fish	Typhlichthys subterraneus		●								●					2
Flatworm	Geocentrophora cavernicola								●							1
Flatworm	Geocentrophora cavernicola		●													1
Gastropod	Anguispira rugoderma		●													1
Gastropod	Aphaostracon pycnus					●										1
Gastropod	Elimia christyi				●							●				2
Gastropod	Floridobia alexander					●										1
Gastropod	Floridobia leptospira					●										1
Gastropod	Fontigens tartarea								●							1
Gastropod	Fumonelix archeri				●											1
Gastropod	Fumonelix wetherbyi		●													1
Gastropod	Gastrodonta fonticula		●						●							2
Gastropod	Glyphyalinia ocoae				●							●				2
Gastropod	Glyphyalinia raderi								●							1
Gastropod	Helicodiscus diadema								●							1
Gastropod	Helicodiscus triodus								●			●				2
Gastropod	Inflectarius magazinensis										●					1
Gastropod	Inflectarius subpalliatus				●											1
Gastropod	Io fluvialis				●				●							2
Gastropod	Leptoxis virgata				●											1
Gastropod	Mesomphix rugeli		●		●											2
Gastropod	Paravitrea septadens								●							1
Gastropod	Pleurocera curta		●													1
Gastropod	Pleurocera showalteri			●												1
Gastropod	Rhodacme elatior	●	●													2
Gastropod	Stenotrema altispira				●				●							2
Gastropod	Stenotrema pilsbryi									●						1
Gastropod	Ventridens decussatus				●				●							2
Gastropod	Vertigo bollesiana		●		●				●							3
Gastropod	Vertigo clappi		●		●				●							3
Isopod	Lirceus bicuspidatus										●					1
Mussel	Alasmidonta marginata		●						●	●						3
Mussel	Alasmidonta undulata											●				1
Mussel	Alasmidonta varicosa			●					●			●	●			4

Mussel	Alasmidonta viridis								●		●	●				3
Mussel	Anodonta heardi					●										1
Mussel	Anodontoides radiatus	●						●								2
Mussel	Cyprogenia aberti									●	●					2
Mussel	Elliptio arca	●			●											2
Mussel	Elliptio arctata	●			●	●										3
Mussel	Elliptio producta												●			1
Mussel	Elliptio purpurella					●										1
Mussel	Elliptio roanokensis											●	●			2
Mussel	Fusconaia askewi						●							●		2
Mussel	Fusconaia lananensis													●		1
Mussel	Fusconaia masoni								●			●				2
Mussel	Fusconaia subrotunda subrotunda		●													1
Mussel	Lampsilis cariosa								●				●			2
Mussel	Lampsilis satura						●							●		2
Mussel	Lasmigona alabamensis	●														1
Mussel	Lasmigona etowaensis	●														1
Mussel	Lasmigona holstonia				●				●			●				3
Mussel	Lasmigona subviridis								●			●				2
Mussel	Obovaria jacksoniana						●			●				●		3
Mussel	Obovaria unicolor							●								1
Mussel	Pleurobema beadleianum							●								1
Mussel	Pleurobema cordatum								●	●						2
Mussel	Pleurobema oviforme		●		●				●			●				4
Mussel	Pleurobema pyramidatum		●													1
Mussel	Pleurobema riddellii						●							●		2
Mussel	Pleurobema rubrum							●	●	●						3
Mussel	Pleuronaia barnesiana				●				●			●				3
Mussel	Potamilus amphichaenus													●		1
Mussel	Simpsonaias ambigua		●													1
Mussel	Strophitus connasaugaensis	●		●	●											3
Mussel	Strophitus subvexus	●					●	●								3
Mussel	Toxolasma corvunculus	●		●												2
Mussel	Toxolasma lividum		●	●					●	●	●					5
Mussel	Toxolasma pullus											●				1
Mussel	Villosa arkansasensis									●						1
Mussel	Villosa nebulosa	●		●	●											3
Mussel	Villosa umbrans	●			●											2

Mussel	Villosa vaughaniana											●				1
Lichen	Alectoria fallacina								●			●				2
Lichen	Arthonia kermesina				●							●				2
Lichen	Arthopyrenia betulicola											●				1
Lichen	Arthopyrenia degelii				●							●				2
Lichen	Graphis sterlingiana											●				1
Lichen	Gyalectidium appendiculatum											●				1
Lichen	Heterodermia appalachensis				●				●			●				3
Lichen	Heterodermia erecta			●					●			●				3
Lichen	Hypotrachyna oostingii								●			●				2
Lichen	Hypotrachyna virginica				●				●			●				3
Lichen	Lecanora masana								●			●				2
Lichen	Lepraria lanata											●				1
Lichen	Melanelia culbersonii								●							1
Lichen	Mycoporum biseptalum											●				1
Lichen	Physcia pseuodspeciosa											●				1
Lichen	Pilophorus fibula											●				1
Lichen	Stereocaulon tennesseense			●	●							●				3
Lichen	Sticta limbata											●				1
Lichen	Usnea angulata											●				1
Lichen	Xanthoparmelia monticola											●				1
Non-Vascular Plant	Acrobolbus ciliatus				●							●				2
Non-Vascular Plant	Anastrophyllum saxicola				●							●				2
Non-Vascular Plant	Bazzania nudicaulis				●				●			●				3
Non-Vascular Plant	Campylopus carolinae											●				1
Non-Vascular Plant	Cephalozia pleniceps var. carolinana											●				1
Non-Vascular Plant	Cephaloziella spinicaulis				●				●			●				3
Non-Vascular Plant	Cheilolejeunea evansii	●		●	●							●	●			5
Non-Vascular Plant	Diplophyllum obtusatum											●				1
Non-Vascular Plant	Diplophyllum taxifolium var. mucronatum											●				1
Non-Vascular Plant	Drepanolejeunea appalachiana				●							●				2
Non-Vascular Plant	Fissidens appalachensis				●							●				2
Non-Vascular Plant	Fissidens hallii											●				1
Non-Vascular Plant	Frullania appalachiana			●	●							●				3
Non-Vascular Plant	Frullania donnellii					●						●				2
Non-Vascular Plant	Homaliadelphus sharpii											●				1
Non-Vascular Plant	Hygrohypnum closteri		●		●							●				3
Non-Vascular Plant	Lejeunea blomquistii			●	●							●				3

Non-Vascular Plant	Lejeunea dimorphophylla											●				1
Non-Vascular Plant	Leptodontium excelsum				●							●				2
Non-Vascular Plant	Leptohymenium sharpii											●				1
Non-Vascular Plant	Leptoscyphus cuneifolius								●							1
Non-Vascular Plant	Lophocolea appalachiana				●							●				2
Non-Vascular Plant	Metzgeria furcata var. setigera											●				1
Non-Vascular Plant	Nardia lescurii		●	●	●				●			●				5
Non-Vascular Plant	Oncophorus raui		●													1
Non-Vascular Plant	Plagiochila austinii				●				●			●				3
Non-Vascular Plant	Plagiochila caduciloba			●	●							●	●			4
Non-Vascular Plant	Plagiochila corniculata				●				●			●				3
Non-Vascular Plant	Plagiochila eurphyllon ssp. echinata				●							●				2
Non-Vascular Plant	Plagiochila sharpii											●				1
Non-Vascular Plant	Plagiochila sullivantii		●		●				●			●				4
Non-Vascular Plant	Plagiochila virginica				●				●			●				3
Non-Vascular Plant	Plagiomnium carolinianum				●							●				2
Non-Vascular Plant	Platyhypnidium pringlei											●				1
Non-Vascular Plant	Pohlia rabunbaldensis			●								●				2
Non-Vascular Plant	Polytrichum appalachianum											●				1
Non-Vascular Plant	Porella japonica ssp. appalachiana				●							●				2
Non-Vascular Plant	Radula sullivantii	●			●							●	●			4
Non-Vascular Plant	Radula tenax				●				●			●				3
Non-Vascular Plant	Radula voluta				●							●				2
Non-Vascular Plant	Riccardia jugata				●							●				2
Non-Vascular Plant	Schlotheimia lancifolia											●				1
Non-Vascular Plant	Scopelophila cataractae		●									●				2
Non-Vascular Plant	Sphagnum flavicomans				●				●			●				3
Non-Vascular Plant	Sphenolobopsis pearsonii				●				●			●				3
Non-Vascular Plant	Taxilejeunea sharpii				●											1
Non-Vascular Plant	Taxiphyllum alternans											●				1
Plant	Abies fraseri				●				●			●				3
Plant	Aconitum reclinatum				●				●			●				3
Plant	Actaea rubifolia								●						●	2
Plant	Agalinis auriculata										●					1
Plant	Agalinis filicaulis					●	●	●								3
Plant	Agalinis navasotensis													●		1
Plant	Agalinis skinneriana						●									1
Plant	Ageratina luciae-brauniae		●													1

Plant	Agrimonia incisa	●				●		●						●		4
Plant	Allium oxyphilum								●							1
Plant	Amorpha ouachitensis									●	●					2
Plant	Amorpha paniculata						●							●		2
Plant	Amsonia ludoviciana						●									1
Plant	Arabis patens								●			●				2
Plant	Aristida simpliciflora	●						●								2
Plant	Asclepias viridula					●										1
Plant	Asplenium x heteroresilens											●				1
Plant	Astragalus soxmaniorum						●							●		2
Plant	Baptisia australis var. aberrans											●				1
Plant	Baptisia megacarpa	●														1
Plant	Bartonia texana													●		1
Plant	Berberis canadensis		●	●					●			●				4
Plant	Boltonia montana								●							1
Plant	Botrychium jenmanii	●			●			●	●			●				5
Plant	Buckleya distichophylla				●				●			●				3
Plant	Calamovilfa arcuata		●							●						2
Plant	Callirhoe bushii									●	●					2
Plant	Calopogon multiflorus					●						●				2
Plant	Calopogon oklahomensis						●	●								2
Plant	Cardamine clematitis			●	●				●			●				4
Plant	Carex brysonii	●														1
Plant	Carex chapmanii					●						●				2
Plant	Carex communis var. amplisquama												●			1
Plant	Carex decomposita	●				●	●	●		●						5
Plant	Carex impressinervia	●						●				●				3
Plant	Carex juniperorum		●													1
Plant	Carex latebracteata									●						1
Plant	Carex polymorpha								●							1
Plant	Carex radfordii			●								●	●			3
Plant	Carex schweinitzii								●							1
Plant	Carex timida									●						1
Plant	Castanea pumila var. ozarkensis						●			●	●					3
Plant	Centrosema arenicola					●										1
Plant	Chelone cuthbertii			●					●			●				3
Plant	Chelone obliqua var erwiniae											●				1
Plant	Cirsium lecontei											●				1

Plant	Cleistesiopsis bifaria	●	●	●	●	●		●	●			●				8
Plant	Clematis addisonii								●							1
Plant	Clematis coactilis								●							1
Plant	Clematis viticaulis								●							1
Plant	Clinopodium dentatum					●										1
Plant	Coelorachis tuberculosa	●				●										2
Plant	Collinsonia tuberosa											●				1
Plant	Collinsonia verticillata		●	●	●								●			4
Plant	Corallorhiza bentleyi								●							1
Plant	Coreopsis latifolia				●							●				2
Plant	Crataegus ashei							●								1
Plant	Crataegus triflora	●						●								2
Plant	Crataegus warneri													●		1
Plant	Ctenium floridanum					●										1
Plant	Cuscuta attenuata									●						1
Plant	Cyperus grayoides						●							●		2
Plant	Cypripedium kentuckiense	●	●				●			●	●			●		6
Plant	Dalea hallii													●		1
Plant	Delphinium alabamicum	●														1
Plant	Delphinium exaltatum								●			●				2
Plant	Delphinium newtonianum									●	●					2
Plant	Delphinium treleasei										●					1
Plant	Desmodium ochroleucum							●								1
Plant	Diervilla rivularis	●			●							●				3
Plant	Dionaea muscipula											●				1
Plant	Dodecatheon frenchii		●													1
Plant	Draba aprica									●	●					2
Plant	Echinacea atrorubens													●		1
Plant	Echinodorus tenellus	●				●			●							3
Plant	Elymus churchii									●	●					2
Plant	Elymus glaucus ssp. mackenziei									●						1
Plant	Eriocaulon koernickianum									●	●					2
Plant	Eupatorium anomalum					●										1
Plant	Euphorbia purpurea								●			●				2
Plant	Eurybia eryngiifolia	●				●										2
Plant	Eurybia saxicastellii		●													1
Plant	Festuca versuta									●						1
Plant	Forestiera godfreyi					●										1

Plant	Fothergilla major	●		●	●							●	●			5
Plant	Gaylussacia brachycera								●							1
Plant	Gentiana latidens											●				1
Plant	Gentiana pennelliana					●										1
Plant	Geum geniculatum				●							●				2
Plant	Glyceria nubigena											●				1
Plant	Grammitis nimbata											●				1
Plant	Gymnocarpium appalachianum								●							1
Plant	Hamamelis ovalis							●						●		2
Plant	Hartwrightia floridana					●										1
Plant	Hasteola robertiorum					●										1
Plant	Hedeoma graveolens					●										1
Plant	Helianthus occidentalis var. plantagineus						●			●						2
Plant	Helianthus smithii	●		●												2
Plant	Heuchera alba								●							1
Plant	Heuchera villosa var. arkansana									●						1
Plant	Hexastylis contracta		●									●				2
Plant	Hexastylis rhombiformis											●				1
Plant	Hexastylis speciosa	●														1
Plant	Hydrophyllum brownei									●						1
Plant	Hymenocallis coronaria												●			1
Plant	Hymenocallis henryae					●										1
Plant	Hymenophyllum tayloriae	●		●								●				3
Plant	Hypericum adpressum									●	●					2
Plant	Ilex collina								●							1
Plant	Illicium parviflorum					●										1
Plant	Ipomoea shumardiana													●		1
Plant	Isoetes microvela											●				1
Plant	Isoetes tennesseensis				●											1
Plant	Juglans cinerea	●	●	●	●			●	●		●	●	●		●	10
Plant	Justicia crassifolia					●										1
Plant	Lachnocaulon digynum	●				●	●	●						●		5
Plant	Leavenworthia aurea									●						1
Plant	Leavenworthia crassa	●														1
Plant	Lesquerella angustifolia									●						1
Plant	Liatris tenuis						●							●		2
Plant	Liatris turgida								●			●				2
Plant	Lilium grayi				●				●			●				3

Plant	Lilium iridollae	●														1
Plant	Lindera subcoriacea	●						●				●				3
Plant	Linum macrocarpum	●						●								2
Plant	Linum westii					●										1
Plant	Litsea aestivalis					●						●				2
Plant	Lotus unifoliolatus var. helleri			●								●				2
Plant	Lysimachia fraseri	●		●	●							●	●		●	6
Plant	Lythrum curtissii					●										1
Plant	Macranthera flammea	●				●		●								3
Plant	Magnolia ashei					●										1
Plant	Marshallia trinervia	●					●	●								3
Plant	Minuartia godfreyi	●														1
Plant	Monotropsis odorata	●	●	●	●				●			●	●			7
Plant	Monotropsis reynoldsiae					●										1
Plant	Myriophyllum laxum	●				●		●				●				4
Plant	Najas filifolia					●										1
Plant	Nemastylis floridana					●										1
Plant	Neviusia alabamensis	●									●					2
Plant	Nolina atopocarpa					●										1
Plant	Packera millefolium			●					●			●				3
Plant	Packera serpenticola											●				1
Plant	Parnassia caroliniana					●										1
Plant	Parnassia grandifolia			●	●	●	●	●	●		●	●				8
Plant	Paxistima canbyi		●						●							2
Plant	Phacelia ranunculacea														●	1
Plant	Phlox buckleyi								●							1
Plant	Phoebanthus tenuifolius	●				●										2
Plant	Physostegia godfreyi					●										1
Plant	Pinckneya bracteata					●										1
Plant	Pinguicula planifolia	●				●		●								3
Plant	Pinguicula primuliflora	●						●								2
Plant	Pityopsis flexuosa					●										1
Plant	Platanthera integra	●				●	●	●				●		●		6
Plant	Poa paludigena								●							1
Plant	Polygala hookeri	●				●		●				●				4
Plant	Polygala leptostachys					●		●								2
Plant	Polymnia cossatotensis									●						1
Plant	Potamogeton hillii								●							1

Plant	Potamogeton tennesseensis				●				●							2
Plant	Prenanthes barbata						●							●	●	3
Plant	Prenanthes crepidinea		●												●	2
Plant	Pteroglossaspis ecristata					●	●	●								3
Plant	Pycnanthemum curvipes			●	●											2
Plant	Pycnanthemum floridanum					●										1
Plant	Pycnanthemum torrei								●			●				2
Plant	Quercus acerifolia									●	●					2
Plant	Quercus arkansana	●					●									2
Plant	Quercus oglethorpensis			●				●					●			3
Plant	Rhexia parviflora	●				●										2
Plant	Rhexia salicifolia	●				●										2
Plant	Rhododendron austrinum	●				●										2
Plant	Rhododendron eastmanii												●			1
Plant	Rhododendron vaseyi											●				1
Plant	Rhynchospora crinipes	●				●		●								3
Plant	Rhynchospora galeana					●						●				2
Plant	Rhynchospora macra					●	●	●				●		●		5
Plant	Rhynchospora pleiantha	●				●						●				3
Plant	Robinia hartwegii											●				1
Plant	Rudbeckia auriculata	●														1
Plant	Rudbeckia heliopsidis	●											●			2
Plant	Rudbeckia scabrifolia						●							●		2
Plant	Rudbeckia triloba var. pinnatiloba				●							●				2
Plant	Ruellia noctiflora	●				●		●								3
Plant	Salix floridana					●										1
Plant	Sarracenia purpurea var. montana											●				1
Plant	Saxifraga caroliniana				●							●				2
Plant	Schisandra glabra	●	●	●			●	●			●					6
Plant	Schoenolirion wrightii						●							●		2
Plant	Schoenoplectus hallii													●		1
Plant	Scutellaria arguta		●		●							●				3
Plant	Scutellaria saxatilis		●		●				●			●				4
Plant	Sedum nevii	●			●											2
Plant	Shortia galacifolia			●	●							●	●			4
Plant	Silene ovata		●	●	●				●		●	●				6
Plant	Silene regia	●									●					2
Plant	Silene subciliata						●							●		2

Plant	Silphium pinnatifidum														●	1
Plant	Silphium wasiotense		●													1
Plant	Solidago albopilosa		●													1
Plant	Solidago ouachitensis									●	●					2
Plant	Solidago plumosa											●				1
Plant	Solidago simulans			●								●				2
Plant	Solidago verna											●				1
Plant	Spiranthes brevilabris													●		1
Plant	Spiranthes eatonii											●				1
Plant	Spiranthes longilabris					●		●				●				3
Plant	Sporobolus curtissii	●				●										2
Plant	Stachys clingmanii				●							●				2
Plant	Streptanthus squamiformis									●						1
Plant	Stylisma abdita					●										1
Plant	Symphyotrichum georgianum	●		●								●	●			4
Plant	Symphyotrichum rhiannon											●				1
Plant	Talinum teretifolium		●	●					●			●				4
Plant	Thalictrum arkansanum									●						1
Plant	Thaspium pinnatifidum	●	●		●							●				4
Plant	Thermopsis fraxinifolia			●	●							●				3
Plant	Thermopsis mollis		●		●				●			●	●			5
Plant	Thermopsis villosa			●								●				2
Plant	Tradescantia ozarkana									●	●					2
Plant	Trifolium virginicum								●							1
Plant	Trillium decumbens	●			●											2
Plant	Trillium delicatum			●												1
Plant	Trillium discolor			●								●	●			3
Plant	Trillium lancifolium			●									●			2
Plant	Trillium pusillum var. ozarkanum									●	●					2
Plant	Trillium simile			●	●							●	●			4
Plant	Trillium texanum													●		1
Plant	Triphora trianthophora var. texensis													●		1
Plant	Tsuga caroliniana				●				●			●				3
Plant	Uvularia floridana	●				●		●								3
Plant	Valerianella nuttallii									●	●					2
Plant	Valerianella ozarkana										●					1
Plant	Valerianella palmeri									●						1
Plant	Verbesina heterophylla					●										1

Plant	Vernonia lettermannii									●						1
Plant	Vicia ocalensis					●										1
Plant	Vitis rupestris		●						●	●						3
Plant	Waldsteinia lobata			●								●	●			3
Plant	Xyris drummondii	●				●	●	●						●		5
Plant	Xyris isoetifolia	●				●										2
Plant	Xyris longisepala	●				●										2
Plant	Xyris louisianica					●	●									2
Plant	Xyris scabrifolia	●				●	●	●						●		5
Amphibian	Aneides aeneus	●	●	●					●			●	●			6
Amphibian	Cryptobranchus alleganiensis		●	●	●				●			●				5
Amphibian	Desmognathus apalachicolae					●										1
Amphibian	Desmognathus folkertsi			●								●				2
Amphibian	Desmognathus organi				●				●			●				3
Amphibian	Lithobates areolatus													●		1
Amphibian	Lithobates capito	●				●						●				3
Amphibian	Notophthalmus perstriatus					●										1
Amphibian	Plethodon caddoensis									●						1
Amphibian	Plethodon chattahoochee											●				1
Amphibian	Plethodon cheoah											●				1
Amphibian	Plethodon fourchensis									●						1
Amphibian	Plethodon hubrichti								●							1
Amphibian	Plethodon kiamichi									●						1
Amphibian	Plethodon kisatchie						●									1
Amphibian	Plethodon ouachitae									●						1
Amphibian	Plethodon punctatus								●							1
Amphibian	Plethodon sequoyah									●						1
Amphibian	Plethodon sherando								●							1
Amphibian	Plethodon virginia								●							1
Amphibian	Plethodon websteri			●				●					●			3
Amphibian	Plethodon welleri				●				●			●				3
Amphibian	Urspelerpes brucei			●												1
Bird	Ammodramus henslowii	●					●		●	●	●			●	●	7
Bird	Grus canadensis pratensis					●										1
Bird	Peucaea aestivalis	●		●		●	●	●		●	●	●	●	●		10
Insect	Agapetus jocassee											●				1
Insect	Allocapnia fumosa								●							1
Insect	Atrytone arogos								●							1

Insect	Atrytonopsis loammi					●						●				2
Insect	Beloneuria georgiana			●												1
Insect	Brachypanorpa jeffersoni				●				●							2
Insect	Calephelis borealis		●						●							2
Insect	Callophrys irus		●		●	●			●			●				5
Insect	Catocala herodias gerhardi								●							1
Insect	Catocala marmorata								●							1
Insect	Cicindela patruela								●							1
Insect	Cordulegaster sarracenia						●							●		2
Insect	Danaus plexippus	●	●	●	●	●	●	●	●	●	●	●	●	●	●	14
Insect	Erora laeta		●		●				●							3
Insect	Erynnis martialis				●	●			●			●				4
Insect	Erynnis persius persius								●							1
Insect	Euchlaena milnei								●			●				2
Insect	Exyra ridingsii					●						●				2
Insect	Gomphus consanguis			●	●							●				3
Insect	Gomphus viridifrons				●				●							2
Insect	Haploperla chukcho							●								1
Insect	Hydroptila wakulla					●										1
Insect	Isoperla sagittata													●		1
Insect	Leuctra szczytkoi						●									1
Insect	Libellula jesseana					●										1
Insect	Manophylax butleri		●													1
Insect	Megaleuctra williamsae								●							1
Insect	Melanoplus nanciae					●										1
Insect	Ophiogomphus edmundo			●	●							●	●			4
Insect	Ophiogomphus howei		●						●			●				3
Insect	Ophiogomphus incurvatus				●							●				2
Insect	Paduniella nearctica										●					1
Insect	Papaipema silphii		●													1
Insect	Peltotrupes youngi					●										1
Insect	Pseudanophthalmus avernus								●							1
Insect	Pseudanophthalmus cordicollis								●							1
Insect	Pseudanophthalmus intersectus								●							1
Insect	Pyrgus wyandot								●							1
Insect	Somatochlora calverti					●										1
Insect	Somatochlora margarita						●							●		2
Insect	Speyeria idalia								●		●	●				3

Insect	Taeniopteryx nelsoni								●							1
Insect	Zale perculta					●										1
Mammal	Corynorhinus rafinesquii	●	●	●	●	●	●	●	●		●		●	●	●	12
Mammal	Glaucomys sabrinus fuscus								●							1
Mammal	Myotis austroriparius	●	●			●	●	●		●	●	●	●	●		10
Mammal	Myotis leibii		●	●	●				●	●	●	●	●		●	9
Mammal	Perimyotis subflavus	●	●	●	●	●	●	●	●	●	●	●	●	●	●	14
Mammal	Podomys floridanus					●										1
Mammal	Sciurus niger shermani					●										1
Mammal	Ursus americanus luteolus						●	●								2
Other Arthropod	Pygmarrhopalites sacer								●							1
Other Arthropod	Escaryus cryptorobius								●							1
Other Arthropod	Escaryus orestes								●							1
Reptile	Clemmys guttata					●										1
Reptile	Crotalus adamanteus	●				●		●				●				4
Reptile	Glyptemys insculpta								●							1
Reptile	Glyptemys muhlenbergii			●	●				●			●				4
Reptile	Gopherus polyphemus			●		●										2
Reptile	Graptemys barbouri					●										1
Reptile	Heterodon simus					●						●				2
Reptile	Pituophis melanoleucus [excluding P. m. lodingi]			●	●				●						●	4
Reptile	Pituophis ruthveni						●							●		2
Reptile	Sceloporus woodi					●										1

Totals	568	91	71	76	113	111	44	58	151	64	42	191	39	46	13

Organismal Group	**Total Species Count**
Lichen	20
Non-Vascular Plant	47
Vascular Plant	245
Flatworm	2
Crustacean	40
Fish	54

Gastropod	1
Isopod	27
Mussel	42
Amphibian	23
Bird	3
Insect	43
Mammal	8
Other Arthropod	3
Reptile	10

Attachment 20

Culvert Effects on Stream and Stream-Side Salamander Habitats

Culvert Effects on Stream and Stream-Side Salamander Habitats

James T. Anderson, Ryan L. Ward, J. Todd Petty, J. Steven Kite, and Michael P. Strager

***Abstract*—Road and stream intersections require a crossing that allows safe passage of water and vehicles. Culverts are normally used when roads cross small streams. Recently, passage of aquatic organisms through culverts has received increased attention. We used a geographic information system (GIS) analysis to determine the degree of salamander habitat fragmentation in Tucker and Randolph counties in West Virginia, USA. We visited state roads with culverts and categorized salamander barriers as complete, partial, or nonbarrier, based on outlet hang, culvert slope, and substrate. Complete barriers occurred at 55.0% of the sites visited and partial barriers at 34.2%. We found that 20.6% of the total stream length in the Dry Fork watershed and 18.4% in the Shavers Fork watershed were isolated by at least a partial barrier. Outlet hang height and the presence (or absence) of streambed substrate were the main determinants of stream salamander passage. Outlet hang was positively correlated with stream gradient and culvert slope. Culverts containing streambed substrate occurred on lower gradient streams, had lower culvert slope, and had a greater width compared to culverts lacking substrate. Solutions to facilitate movement of salamanders and other aquatic organisms are needed to maintain stream connectivity and provide mitigation opportunities.**

***Index Terms*—Stream salamanders, culverts, habitat fragmentation, roads, streams, passage.**

I. INTRODUCTION

Roads are a necessary component of human lives and a prominent feature on the landscape. The need for roads is not likely to change, and therefore as wildlife managers and environmental stewards we should strive to minimize their impacts on wildlife and their ecosystems. Practitioners of stream restoration have begun to develop ways to maintain stable road crossings and functioning streams [1]. However, the field of culvert installation to maintain geomorphic stability and provide passage of fish and other aquatic organisms is still in its infancy [2].

Salamanders have received little consideration for passage through culverts [2], [3]. Most studies focus on the passage of fish, including the development of computer software to analyze culvert passage issues [2], [4], [5]. Excessive outlet hang of culverts is a common condition that blocks fish passage [2], [6]; however, culvert slope also can block fish passage [5]. The culvert outlet bottom should be below the outlet pool to prevent hanging barriers [7]. However, fish are able to use the outlet pool to swim and attain a sufficient velocity to overcome modest outlet hang [8]-[10]. Amphibians are weak swimmers compared to fish [11]. The reduced swimming ability of salamanders would most likely inhibit their ability to make use of outlet pools as areas to build up speed for jumping out of the water although salamanders on land can jump short distances to avoid predators [12].

Adult Axolotls (*Ambystoma mexicanum*) have lower swimming ability than most fishes [13]. Lower swimming abilities suggest that high water velocities through culverts would have a greater impact on salamanders than on fish. During normal runoff conditions, water velocities in streams are typically 0–0.9m/sec near the channel bed and stream margins where most aquatic organisms live and travel [2]. Differences in water velocity in culverts compared to natural channels are likely to impede salamander passage [11].Flow velocities in culverts with gradients as low as 1–2% may exceed 1.2–1.5m/sec under normal runoff conditions without even constricting the channel width [2]. Velocities in culverts exceed extreme velocities in the natural channel that average 0.9–1.8m/sec during bankfull flows [2]. Terrestrial capabilities of salamanders could possibly allow them to navigate through or around culverts, but leaving their preferred habitat and crossing roads may make them vulnerable to predators and automobile mishaps [11].

Culverts need to be placed so the culvert bottoms mimic natural streambeds [14], because the physical nature of the bed material in a stream may be the most biologically significant factor affecting stream functions [15]. Salamanders use the channel substrate for refuge and foraging [16]. Culvert hang and slope as described above is also a major factor contributing to passage issues. Newly constructed roads that cross streams need to be built in a manner to not restrict any form of aquatic life movement, not just fish [3].

Previous research in the Appalachians has found that the presence of roads with culverts that were designated as being complete barriers to stream salamander movement was an important factor in dictating differences in salamander abundance, diversity, and richness at both the stream-level and the reach-level (i.e., above and below culverts) [3]. Indeed, abundance of Northern Two-lined (*Eurycea bislineata*), Appalachian Seal (*Desmognathus monticola*), Northern Spring (*Desmognathus fuscus*), and Mountain

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Dusky (*Desmognathus ochrophaeus*) Salamanders were all impacted by roads. All of these species except for Northern Two-lined Salamanders were negatively influenced by stream culverts. However, this study did not evaluate the extent of the barrier impact problem on amphibians in the Appalachians.

The objectives of our study were to:

1) Determine the extent of habitat fragmentation for salamanders by roads and culverts in the lower Shavers Fork watershed and Dry Fork watershed, West Virginia, USA;
2) Determine installation parameters of corrugated steel pipe culverts that will prevent excessive outlet hang and best promote the passage of stream and stream-side salamanders;
3) Determine installation parameters of circular and pipe arch culverts constructed of corrugated steel pipe that will allow for the retention of bedload material and best promote the passage of stream and stream-side salamanders.

II. MATERIALS AND METHODS

A. Study Area

We conducted culvert surveys in the lower Shavers Fork and the Dry Fork watersheds of the upper Cheat River basin (Fig. 1). The study area was located in Randolph and Tucker counties in eastern West Virginia [3], [5].

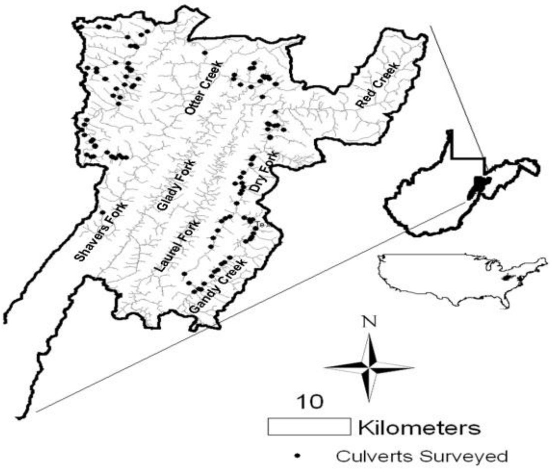

Fig. 1. Map of study area in the lower Shavers Fork and Dry Fork watersheds, West Virginia, USA 2003.Circles indicate state culverts where surveys were conducted.

Shavers Fork flows to the town of Parsons, West Virginia, where it combines with the Black Fork to form the Cheat River. Shavers Fork is a cold and cool water fishery with most tributaries consisting of high gradient mountain streams. Surveys were conducted in the lower portion of the watershed, which contained an extensive state road network. The U.S. Forest Service or private landowners owned most roads in the upper portion of the watershed, and this area was excluded for this reason.

The Dry Fork flows near Parsons where it combines with the Blackwater River to form the Black Fork. Dry Fork maintains a cool-water fishery and a cold-water fishery in some sections. Major tributaries to Dry Fork include Gandy Creek, Laurel Fork, Glady Fork, and Red Creek. Laurel Fork and Glady Fork were excluded from the study due to a lack of state owned roads within their sub-watersheds. Red Creek was excluded from the study due to poor water quality. High gradient mountain streams characterized minor tributaries to Dry Fork and Gandy Creek. Otter Creek empties into lower Dry Fork from the Otter Creek Wilderness area.

The average winter temperature in the study area is –0.5˚C and the average summer temperature is 20.1˚C [17], [18]. Average annual rainfall in the study area is 116cm [17], [18]. Prevailing winds in the study area occur from the northwest and west [17], [18]. Elevations ranged from 518m to 1,472m. The most abundant geologic map units were the Pottsville group, Mauch Chunk group, Hampshire formation, and Chemung group [19].

B. Methods

Our study was conducted in three phases. First, we used geographic information system (GIS) analysis to create working maps for field crews. Next, we visited each culvert site and performed field surveys. Last, we conducted data analyses to interpret collected data.

We used ArcMap GIS ver. 8.2 software (Environmental Systems Research Institute, Redlands, California, USA) to determine the location of streams that drain at least 40.5ha (100 acres). Then we added a layer of roads to find stream and road intersections. We used these layers to construct maps to assist field crews in finding stream crossings. Streams listed as impaired in 2003 according to the Clean Water Act Section 303d were excluded (West Virginia Department of Environmental Protection, unpubl. data). Streams with drainage areas less than 40.5ha, although ecologically important, were excluded because their small size offered fewer significant mitigation opportunities.

We conducted culvert surveys from June to November of 2003 following methods outlined in [5]. We visited each site to determine the crossing type (culvert, bridge, or ford). Only sites with culverts were surveyed. We recorded the type of each culvert, construction materials, length, and diameter or height and width. The active channel of the stream was defined as the portion of the channel that was lacking vegetation due to frequent water flows [6]. We took four measurements of the active channel width upstream of the culvert, and we took four measurements of bankfull width and bankfull depth at a typical riffle. We determined the length of stream reaches to be surveyed by multiplying the mean active channel width by 30. We used a minimum length of 30m and a maximum length of 100m to limit reach lengths. The culvert generally was located in the center of each stream study reach. Where a culvert occurred near a stream mouth, we surveyed the entire downstream reach if it was not long enough to meet the total desired reach length.

We surveyed the longitudinal profile of the stream in the study reach from the head of the first riffle to the head of the last riffle. We recorded elevations at the following points:

head of upstream riffle, inlet, outlet, deepest part within 2m of outlet, deepest part of outlet pool, tailwater control, the active channel margin at the tailwater control, head of downstream riffle, and additional slope breaks [6]. We collected additional habitat measurements, including estimation of the percent canopy of the overstory, shrub, and herbaceous layers for each bank both upstream and downstream, presence or absence of continuous suitable salamander (>40mm diameter) substrate above, below, and inside culverts, and completion of Environmental Protection Agency (EPA) habitat assessment forms [20] for up and downstream.

C. Data Analysis

We conducted surveys on all culverts on state roads and the full data set was used to analyze the barrier effects as a whole on the landscape. We considered any culvert with an outlet hang over 0.10m a complete barrier for salamanders [3]. Outlet hangs of 0.05–0.10m were considered partial barriers. Culverts with outlet hangs under 0.05m, but lacking continuous substrate were considered partial barriers. Only if a culvert had an outlet hang under 0.05m and continuous substrate, did we consider it passable (Fig. 2). We used ArcMap GIS ver. 9.0 software (Environmental Systems Research Institute, Redlands, California, USA) to determine the length of stream reaches fragmented by complete and partial barrier culverts. We excluded Otter Creek from analysis of fragmentation because it is wilderness area and lacks an active road system. We used a G-test of association to test if the barrier types observed differed from expected values and for comparison of barrier types between watersheds [21].

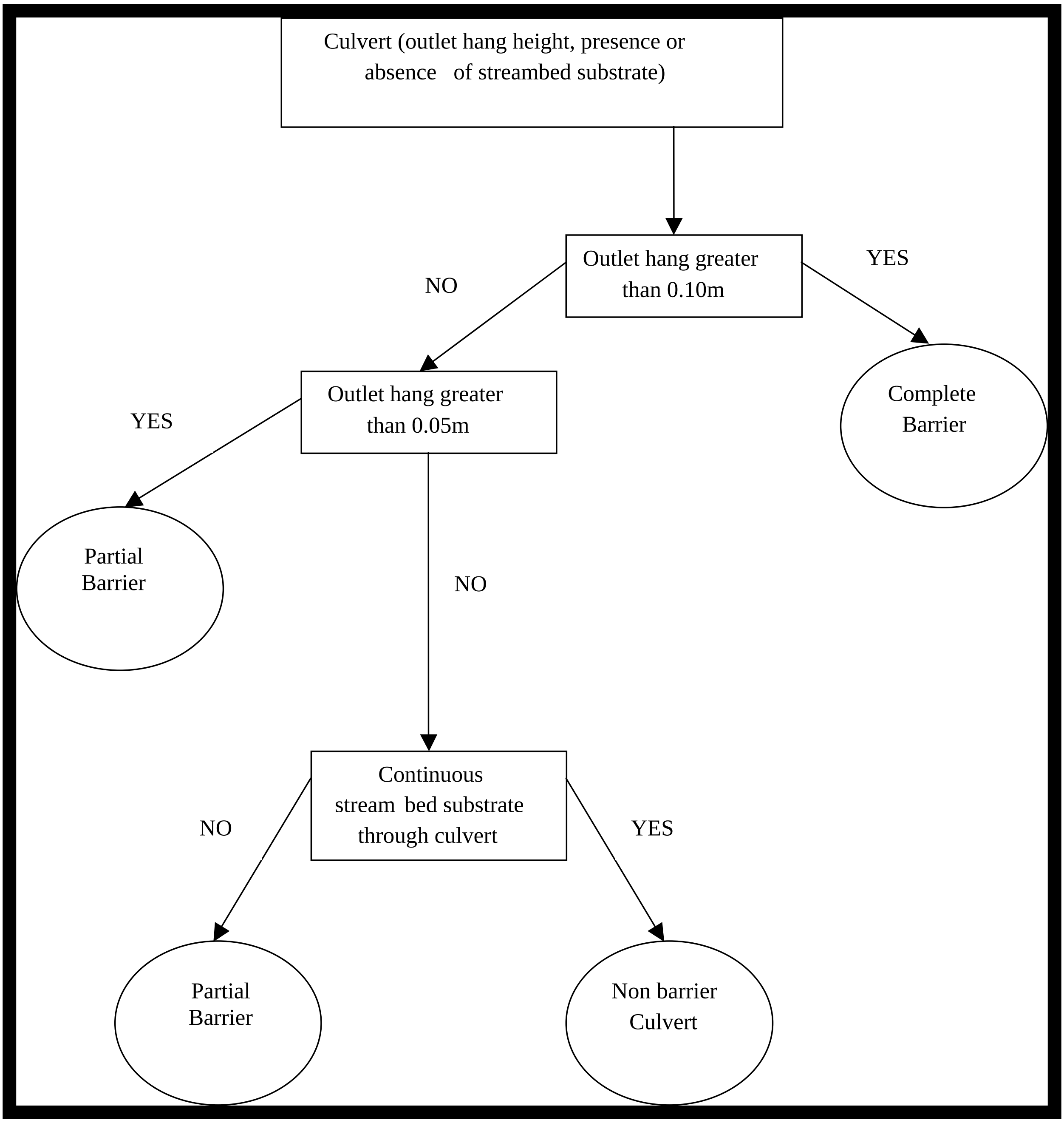

Fig. 2. Decision tree used to determine barrier status of culverts for stream salamanders in the lower Shavers Fork and Dry Fork watersheds, West Virginia, USA. Culverts were classified as complete barriers ($n = 66$), partial barriers ($n = 41$), and nonbarriers ($n = 13$).

Corrugated steel pipe was the most common construction material used for culverts in the study area. Because of their numbers, analyses of culvert parameters were restricted to culverts constructed of corrugated steel pipe. Some stream crossings contained multiple culvert barrels. Because of the low number of sites with multiple barrels, they were removed from analyses of culvert parameters. We used linear regression to examine relations between hang height and stream gradient, culvert length, and culvert slope. We conducted analyses of bedload retention only on sites with continuous substrate upstream of the culvert. This restriction was intended to rule out bedrock streams that were bedload limited. We used a G-test to compare circular and pipe arch culverts, and their ability to retain stream bedload [21]. We used t-tests, assuming equal variances, to compare culvert variables for culverts with and without continuous substrate. We analyzed the variables of circular culverts separately from pipe arch culverts, because different shapes likely influenced hydraulic forces. We considered tests to be significant at $P < 0.05$ and did not transform data because normality was not violated [22]. Comparisons we conducted included culvert variables (slope and diameter/width), stream variables (gradient), and ratios of culvert variables versus stream variables (culvert diameter/width versus active channel width, culvert slope versus stream gradient, and culvert cross-sectional area versus bankfull cross-sectional area).

III. RESULTS

A. *Culvert Surveys*

A total of 120 culvert sites was surveyed in the Dry Fork ($n = 68$) and Shavers Fork ($n = 52$) watersheds. Single culverts occurred at 116 sites, while the remaining four sites had double culverts. Circular culverts were the most common type, occurring at 66 sites. Pipe arch culverts were the second most abundant type, occurring at 36 sites. Box culverts occurred at 13 sites. Combinations of box and circular culverts occurred at five sites. These combined culverts consisted of old stone box culverts that were lengthened with corrugated steel or concrete pipe culverts when the roadway was widened. Corrugated steel pipe was the most common construction material occurring at 94 sites, and concrete was used at 20 sites. The remaining six sites were made of stone or stone and corrugated steel pipe combinations.

B. *Habitat Fragmentation*

Culverts were likely to create barriers to salamanders as salamander movement conditions were classified as complete barriers at 55.0%, partial barriers at 34.2%, and unrestricted passage at 10.8% of culverts surveyed (Fig. 3; Ward 2005) ($n = 120$, $G = 38.90$, $df = 2$, $P < 0.001$). Culverts in the Shavers Fork watershed were more likely to be complete barriers than in the Dry Fork watershed (Fig. 3) ($G = 14.32$, $df = 2$, $P < 0.001$). Barriers isolated 20.6% of the total lengths of stream draining >40.5ha in the Dry Fork watershed and 17.4% in the Shavers Fork watershed (Table I).

C. *Outlet Hang*

Hang height was correlated with stream gradient for corrugated steel pipe culverts ($n = 90$, $R^2 = 0.185$, $P < 0.001$) (Fig. 4). A correlation also existed between hang height and culvert slope ($R^2 = 0.096$, $P = 0.002$). A weak correlation was found between culvert length and hang height ($R^2 = 0.056$, $P = 0.02$).

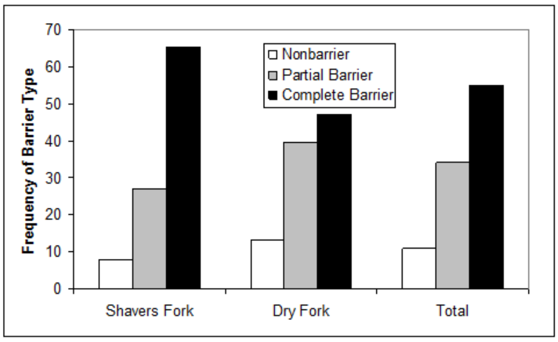

Fig. 3. Frequency of barrier categories for salamanders in the lower Shavers Fork ($n = 52$) and Dry Fork ($n = 68$) watersheds, West Virginia, USA 2003.

TABLE I: LENGTHS OF STREAM AFFECTED BY BARRIER CULVERTS LOCATED ON STATE ROADS IN THE DRY FORK AND SHAVERS FORK WATERSHEDS, WEST VIRGINIA, USA 2003

		Isolated Stream Segments (km)		
	Total Stream Length (km)	**Complete Barriers**	**Partial Barriers**	**Total**
Dry Fork	341.5^{a}	42.7 (12.5%)	27.6 (8.1%)	70.3 (20.6%)
Lower Shavers Fork	276	31.9 (11.6%)	18.7 (6.8%)	50.6 (17.4%)

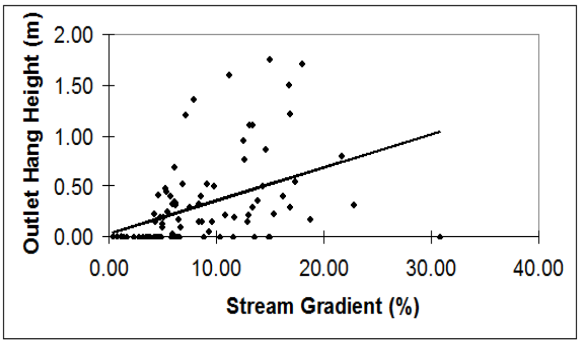

Fig. 4. Linear regression showing the relation between outlet hang height and stream gradient for 116 single barrel culverts in the Dry Fork and Shavers Fork watersheds, West Virginia, USA 2003.

D. *Continuous Substrate*

Of the 120 culverts surveyed, upstream reaches had continuous substrate in 87.3% of surveyed sites, and downstream reaches had continuous substrate in 85.6% of the sites. Only 17.9% of the sites had continuous substrate throughout the entire culvert length (Fig. 5). Culverts tended to create breaks in the stream channel material ($n = 120$, $G = 61.49$, $df = 1$, $P < 0.001$).

We performed analyses on a total of 53 circular culverts (9 with continuous substrate) and 29 pipe arch culverts (5 with continuous substrate). No difference was found between the proportion of circular culverts with substrate (17.0%) and the proportion of pipe arches with substrate (17.2%) (n = 82, G = 0.31, df = 1, P = 0.54).

Culvert slopes were lower for culverts with continuous substrate compared to those lacking substrate (Table II). Culvert diameters were greater for culverts with continuous substrate compared to those without continuous substrate (Table II). Culverts with continuous substrate occurred on streams with significantly less gradient than culverts without continuous substrate (Table II). There was no difference for stream gradient of pipe arches, but pipe arches with continuous substrate were wider than culverts lacking continuous substrate (Table III).

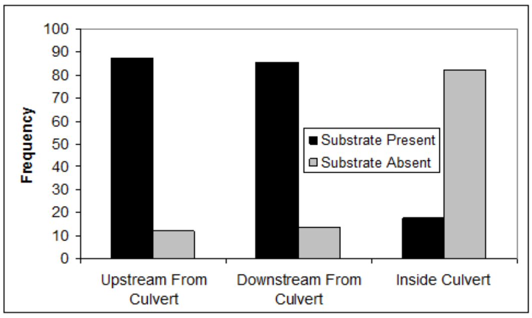

Fig. 5. Graph showing frequency distribution of areas with continuous substrate at culvert sites for 120 culverts in the Dry Fork and Shavers Fork watersheds, West Virginia, USA 2003.

TABLE II: RESULTS OF ANALYSES ON RETENTION OF STREAMBED SUBSTRATE PERFORMED ON 53 SINGLE, CIRCULAR CULVERTS CONSTRUCTED OF CORRUGATED STEEL PIPE IN THE DRY FORK AND SHAVERS FORK WATERSHEDS, WEST VIRGINIA, USA 2003

	No Substrate		**Substrate**			
Variable	**Mean**	**SE**	**Mean**	**SE**	***t*-test**	***P*-value**
Culvert Diameter vs. Active Channel Width	0.60	0.05	0.66	0.07	-0.61	0.271
Culvert Slope vs. Stream Gradient	0.69	0.06	0.51	0.09	1.43	0.079
Bankfull X-sect. vs Culvert X-sect. Area	1.12	0.17	1.20	0.51	-1.12	0.134
Culvert Slope[a]	6.81	0.58	3.01	0.81	2.84	0.003
Stream Gradient[a]	10.86	0.91	6.56	1.46	2.03	0.024
Culvert Diameter[a]	1.15	0.06	1.87	0.30	-3.86	0.001

[a]significant alpha level = 0.05.

TABLE III: RESULTS OF ANALYSES ON RETENTION OF STREAMBED SUBSTRATE PERFORMED ON 29 SINGLE, PIPE ARCH CULVERTS CONSTRUCTED OF CORRUGATED STEEL PIPE IN THE DRY FORK AND SHAVERS FORK WATERSHEDS, WEST VIRGINIA, USA 2003

	No Substrate		**Substrate**			
Variable	**Mean**	**SE**	**Mean**	**SE**	***t*-test**	***P*-value**
Culvert Width vs. Active Channel Width	0.93	0.06	1.07	0.13	-0.86	0.1982
Culvert Slope vs. Stream Gradient	0.74	0.08	0.47	0.13	1.37	0.0906
Bankfull X-sect. vs Culvert X-sect. Area	1.70	0.23	1.99	0.29	-0.57	0.2872
Culvert Slope	4.54	0.50	3.18	1.21	1.11	0.1383
Stream Gradient	7.21	0.83	5.79	1.12	0.75	0.2296
Culvert Width[a]	1.88	0.13	2.64	0.34	-2.39	0.0120

[a]significant alpha level = 0.05.

IV. DISCUSSION

A. Habitat Fragmentation

The majority of culverts surveyed were complete or partial barriers to stream salamanders (89%). These sites most likely prevented the movement of salamanders at all or most flow conditions. Complete barriers occurred at a greater frequency than expected from chance alone. A higher percentage of culverts in the lower Shavers Fork watershed were complete barriers compared to the Dry Fork watershed. One possible explanation for this result is the number of high gradient streams in the lower Shavers Fork watershed because of steeper topography. The Dry Fork watershed had more low gradient streams, especially in the Gandy Creek subwatershed. Extra consideration must be given to placing culverts on high gradient streams (>8%). Upstream movement of salamanders and connectivity of salamander habitats are important for the persistence of populations over time [23], [24].

Culverts isolated headwater streams from downstream areas. Headwater streams are the most important habitat for stream salamanders and have the highest densities of salamanders [25], [26]. In the Dry Fork watershed, 20.6% of the total stream length was isolated from colonization sources. In the Shavers Fork watershed, 18.4% of the total stream length was isolated. Isolated streams consisted of low order headwater streams separated from the mainstem of each river. Salamanders move into these areas to exploit resources available in less hydraulically stable streams lacking fish populations [11].

Culverts with hang heights of ≥0.10m were considered complete barriers to stream salamanders. Indeed, this hang height has an influence on salamander communities [3]. We hypothesized that small body sizes and weak swimming abilities probably prevent stream salamanders from overcoming large outlet hangs. Fish can overcome larger hang height because of their ability to use the outlet pool to gain speed for jumping [8]-[10]. Culverts with hang heights of 0.05-0.10m were considered partial barriers. These hang heights were only estimates we made for the passage of adult stream salamanders. We are confident that stream salamanders cannot overcome hang heights ≥0.10m, but lower heights possibly might be overcome if the right conditions existed. Passage for larval salamanders would likely still be prevented at any outlet hang height. Older life stages of salamanders are more likely to undergo upstream movements [27]. However, many salamander species have larval stages longer than a year [12], and salamanders often undergo upstream movement to find suitable wintering locations [28], [29]. Hang heights under 0.05m were considered passable by adult salamanders even though larval stream salamanders would not be likely to overcome any hang at the outlet.

Culverts that prevent up and downstream movements of salamanders affect the structure of populations and the ability of individuals to locate wintering sites [27]-[29]. Also, some salamanders use streambeds as movement corridors [30]. Workers should try to minimize outlet hang in crossing structures. This minimization may require the building of a bridge on a high gradient stream and avoiding the use of a culvert altogether.

B. *Substrate Effects*

The presence of continuous streambed substrate throughout the culvert was required to categorize a culvert as a nonbarrier. Streambed material creates variations in the flow velocity that allow salamanders to move upstream [11]. To pass a culvert the salamander had to be able to enter the culvert barrel and then negotiate the entire culvert length. Retention of streambed material allows salamanders to overcome high velocities found inside culverts.

Culverts in the study area are serving poorly to retain bed material and prevent breaks in the substrate. Only 17.9% of the sites visited contained continuous substrate through the culvert while most upstream reaches (87.3%) and downstream reaches (85.6%) had continuous substrate. These results show a failure of culverts to mimic natural channels and show a need for better culverts and installation methods. If dimensions and placement of corrugated steel pipe culverts can be done properly to prevent breaks in the stream substrate, they may provide a low cost alternative to other options such as bridges and concrete culverts that provide adequate substrate.

Circular culverts with continuous substrate had larger diameters than culverts lacking continuous substrate. A similar trend was observed in the width of pipe arches. A larger diameter or width prevents pooling at the inlet of a culvert and subsequent deposition of bed material before entering the culvert [2]. Within the study area 91% of aggraded reaches at culverts were at least partially caused by low conveyance [31]. Small diameters or widths also constrict the flow of streams, which can cause increased water velocity [2]. Wider culverts better simulate natural stream conditions [2]. Larger culverts also prevent the failure of fill dirt used in the roadbed that can be a source of sedimentation in streams [32]. Wide culverts tend to be found on larger streams, and these large streams tend to be lower in gradient and able to successfully retain substrate. More attention should be given to smaller streams because of their overall importance and the historical lack of consideration given to them.

C. *Management Implications*

Culverts can be modified to benefit aquatic organisms [10]. Velocities have been slowed with the addition of corrugations and baffles inside culverts, which increase roughness [6]. Baffles can improve fish passage and show good durability [8], [10]. Problems with baffles include high cost, difficulty in fabrication, sedimentation, debris jams, icing, and increased turbulence through the culvert [7], [10], [14]. The increased turbulence created by baffles may be negative for salamanders. However, if baffles trap some sediment this might provide a suitable surface for salamanders to use during passage. Weirs can be used to adjust stream gradients at the inlet and outlets of culverts, compensating for large drops and hydraulic forces [6]. If water is pooled into the culvert outlet this would help salamanders enter culverts.

Most research on culverts and barriers has concentrated on fish. Swimming abilities of salamanders is relatively unknown compared to most fish species. More data on the abilities of salamanders would better allow for the analysis of barriers. Future research needed includes the effects of fragmentation on stream salamander populations and population genetics. Also more studies are needed on the movement of stream salamanders. Detailed studies are needed on distances moved, reasons for movement, and timing of movement for different life stages of salamanders. Research on culvert designs should include ways to increase roughness and preventing increased water velocities. New types of culvert are needed to accommodate passage of a wider range of aquatic organisms. When designing new culverts, consideration should be given to cost and ease of installation. Development of methods to retrofit existing culverts to eliminate passage problems would provide a more cost effective alternative to replacement of culverts that are still structurally sound.

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Attachment 21

Population trends in *Vermivora* warblers are linked to strong migratory connectivity

Population trends in *Vermivora* warblers are linked to strong migratory connectivity

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Migratory species can experience limiting factors at different locations and during different periods of their annual cycle. In migratory birds, these factors may even occur in different hemispheres. Therefore, identifying the distribution of populations throughout their annual cycle (i.e., migratory connectivity) can reveal the complex ecological and evolutionary relationships that link species and ecosystems across the globe and illuminate where and how limiting factors influence population trends. A growing body of literature continues to identify species that exhibit weak connectivity wherein individuals from distinct breeding areas co-occur during the nonbreeding period. A detailed account of a broadly distributed species exhibiting strong migratory connectivity in which nonbreeding isolation of populations is associated with differential population trends remains undescribed. Here, we present a range-wide assessment of the nonbreeding distribution and migratory connectivity of two broadly dispersed Nearctic-Neotropical migratory songbirds. We used geolocators to track the movements of 70 *Vermivora* warblers from sites spanning their breeding distribution in eastern North America and identified links between breeding populations and nonbreeding areas. Unlike blue-winged warblers (*Vermivora cyanoptera*), breeding populations of golden-winged warblers (*Vermivora chrysoptera*) exhibited strong migratory connectivity, which was associated with historical trends in breeding populations: stable for populations that winter in Central America and declining for those that winter in northern South America.

animal tracking | conservation | geolocator | limiting factor | migration

Populations of migratory species can be limited by factors throughout their annual cycle. The degree to which spatially isolated breeding populations use geographically distinct areas during the nonbreeding period (i.e., migratory connectivity) affects the potential for regionally specific factors to influence population trends (1–3). Tracking migratory animals to link breeding populations with nonbreeding areas has primarily focused on the migratory behaviors of large mammals and large birds, which have been studied for decades, and even centuries (4–9). However, following recent technological advances, it is now possible to track all but the smallest migratory species across time and space (10–12). The value of tracking species throughout the annual cycle is manifold. Identifying the migratory pathways by which animal populations navigate between breeding and nonbreeding areas can reveal population-level differences in route or space use that may explain differential breeding population trends (13) or signal tradeoffs in life-history strategies (14–16). In migratory birds, the distribution of breeding populations during the nonbreeding period has the potential to be the primary driver of population trends as many long-distance migrant species spend more time on nonbreeding sites than in any other location during the annual cycle (17). Furthermore, environmental conditions experienced during the nonbreeding period can have both direct (18) and indirect effects (19) on individuals, and can consequently influence population trends. As such, describing the spatial structure and level of dispersion of a migratory species during the nonbreeding period can identify potential areas that may limit some populations but not others (20–22), provide insight into the evolutionary history of migratory species (23–25), and aid in the identification of important areas that may be targeted for conservation (18, 21, 26–30).

Despite a growing body of information on the behaviors and connectivity of migratory birds, detailed range-wide studies investigating the nonbreeding distribution and migratory connectivity of entire species remain rare (20, 21). Weak migratory connectivity is most commonly reported in studies of long-distance migratory bird species worldwide (31–35). Weak connectivity results in nonbreeding areas that are inhabited by

Significance

Identifying drivers of population trends in migratory animals is difficult due to their reliance on different geographic regions throughout the annual cycle. Populations of Nearctic–Neotropical migratory birds are often thought to be limited by spatial variation in factors affecting reproduction and survival during the breeding season. We tracked individual songbirds from a two-species complex of New World warblers and discovered unequivocal evidence of a system in which strong associations between breeding areas and nonbreeding areas (i.e., migratory connectivity) is concordant with breeding population trends. The strong migratory connectivity we documented is associated with differential rates of land-use change in population-specific nonbreeding areas. Our results suggest that other migratory species with similar population trends may also exhibit strong migratory connectivity.

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Data deposition: Data used for this study are freely and publicly available on the Data Repository for the University of Minnesota (https://conservancy.umn.edu/handle/11299/193202).

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individuals from multiple, widely dispersed breeding populations. Strong migratory connectivity, resulting in geographic isolation during the nonbreeding period, is required for differential population trends of geographically distinct breeding populations to be driven by factors away from the breeding grounds. Although the theoretical implications of strong connectivity have been addressed and discussed (2), rarely are species' breeding population trends decisively linked to individual populations' occurrence at isolated nonbreeding areas, and this information can be particularly important for the conservation of declining and threatened species. Identifying species with populations that might be independently limited by factors outside of the breeding period will contribute to the understanding of the ecological and evolutionary implications of strong migratory connectivity, and why it appears to be uncommon among migratory birds.

We investigated the migratory connectivity of *Vermivora* wood-warblers (Parulidae), a species complex composed of two extant species of obligate Nearctic-Neotropical migrant warblers that are extremely closely related (36). Golden-winged warblers (*Vermivora chrysoptera*) and blue-winged warblers (*Vermivora cyanoptera*) breed and migrate throughout deciduous forests of eastern North America and occur throughout Central America, with golden-winged warblers also occurring in northern South America during the nonbreeding period, and recent evidence suggests golden-winged warblers may exhibit strong, range-wide migratory connectivity (29, 37). On the breeding grounds, golden-winged warblers and blue-winged warblers have overlapping distributions and regularly hybridize to produce viable young (38). Recent genomic evidence suggests overlap and hybridization has occurred for >1,000 y and that these two species may constitute a single, polymorphic species with differences in their genomes primarily associated with different plumage traits (36). That said, detailed information on the genetic structure of *Vermivora* populations is lacking (36, 39). Furthermore, there is little evidence of costs to producing hybrid young in this system (38, 40). Both species of *Vermivora* and two recognized hybrid phenotypes breed in a similar habitat, often with overlapping territories; sing songs with overlapping characteristics; and exhibit nearly identical foraging and reproductive strategies (41, 42).

Despite remarkable genetic, behavioral, and natural history similarities, regional populations of *Vermivora* have experienced starkly contrasting population trajectories since standardized monitoring began in 1966 (43, 44). Golden-winged warblers breeding throughout the Great Lakes region have maintained historical abundances, whereas golden-winged warblers breeding throughout the Appalachian Mountains region have declined steadily (44) (Fig. 1 *C* and *D*). To date, declines in the Appalachian Mountains have amounted to a loss of 98% of historical abundance and resulted in regional extirpations from many areas where golden-winged warblers were once common (41). Hypotheses about the cause of these declines have focused on breeding-grounds factors, namely, habitat loss and hybridization (i.e., genetic swamping), as the primary drivers of declines in Appalachian Mountains populations of golden-winged warblers (41). However, habitat loss and hybridization fail to parsimoniously explain the stationary population trends of Great Lakes populations of golden-winged warblers and the stationary population trends of blue-winged warblers throughout their distribution, including in the Appalachian Mountains, where they co-occur with historically declined populations of golden-winged warblers (42). Previous investigations into the migratory connectivity of *Vermivora* warblers have focused exclusively on golden-winged warblers and are equivocal. Assessments of stable isotopes in feathers of golden-winged warblers collected during the nonbreeding period showed a possible overlap between Appalachian and Great Lakes populations in Central America (46), whereas a smaller scale, light-level geolocator study found no evidence of nonbreeding population overlap between golden-winged warblers at Great Lakes and Appalachian breeding sites (29). Populations of blue-winged warblers, including those breeding sympatrically with declining golden-winged warblers in the Appalachian Mountains region, have remained numerically stable (44) (Fig. 1 *E*–*G*). This suggests that the limiting factor primarily driving declines of Appalachian golden-winged warblers is likely experienced somewhere outside the breeding period at a time or location that exclusively affects golden-winged warblers that breed in the Appalachian Mountains.

Here, we present the results of a large, range-wide study (Fig. S1) tracking individual songbirds and describe the migratory connectivity of two hybridizing migratory species. We used light-level geolocators (hereafter, geolocators; *Materials and Methods* and *SI Materials and Methods*) to track individual *Vermivora* warblers throughout their annual cycle and determine links between breeding and nonbreeding areas. Based on differences in breeding population trends (Fig. 1) that are not explained by breeding-grounds factors, we predicted that golden-winged warblers would exhibit strong migratory connectivity, with declining populations occurring disparately from numerically stable populations. If there is strong migratory connectivity and isolation among populations during the nonbreeding period, we further predicted that nonbreeding areas used by historically declining populations will have experienced disproportionate rates of forest loss that coincide with population trends. We discuss the implications of the observed migratory connectivity of *Vermivora* warblers in ecological and evolutionary frameworks. Lastly, we identify species that share similar nonbreeding distributions with *Vermivora* and exhibit varied regional population trajectories akin to those seen in golden-winged warblers that we hypothesize may have similarly strong migratory connectivity driving those species' regional population trends.

Results and Discussion

Geolocator-marked golden-winged warblers ($n = 41$) occurred at sites from Guatemala and southern Mexico to northern Venezuela during the nonbreeding period (Fig. 2*A*). Individuals from historically stable Great Lakes breeding populations [bird conservation region (BCR) S12: Boreal Hardwood Transition; *Materials and Methods* and Fig. 1] were dispersed broadly and almost exclusively throughout Central America during the nonbreeding period [28 of 29 (97%); Fig. 2 *B* and *C*], although one individual from a breeding site in central Ontario, Canada, occurred in northern South America (Fig. 2*A*). Golden-winged warblers from historically declining breeding populations in the Appalachian Mountains (BCR S28: Appalachian Mountains, $n = 12$) occurred at sites exclusively in northern South America, primarily in northern Venezuela (Fig. 2 *D* and *E*), during the nonbreeding period. Blue-winged warblers ($n = 25$), which exhibit historically stable population trends throughout their breeding distribution (BCRs S23, S24, and S28: Prairie Hardwood Transition, Central Hardwoods, and Appalachian Mountains, respectively; Fig. 1), occurred almost exclusively in Central America during the nonbreeding period [24 of 25 (96%); Fig. 2*F*]. Only one blue-winged warbler from a northern Appalachian Mountains breeding population occurred in northern South America. Phenotypic hybrids ($n = 4$) from breeding populations in the Great Lakes region ($n = 2$) occurred in northern Central America during the nonbreeding period (Fig. 3*B*), whereas hybrids from breeding populations in the Appalachian Mountains ($n = 2$) occurred in northern South America (Fig. 3*C*). Golden-winged warblers from Great Lakes breeding populations and blue-winged warblers overlapped extensively in Central America during the nonbreeding period (Fig. 2 *B*, *C*, and *G*–*J* and Fig. S3). However, historically declining Appalachian Mountains populations of golden-winged warblers were isolated from historically stable populations of both golden-winged warblers and blue-winged warblers during the nonbreeding period (Fig. S2).

Regional breeding populations of blue-winged warblers (i.e., populations identified by their BCRs) did not occur in isolation

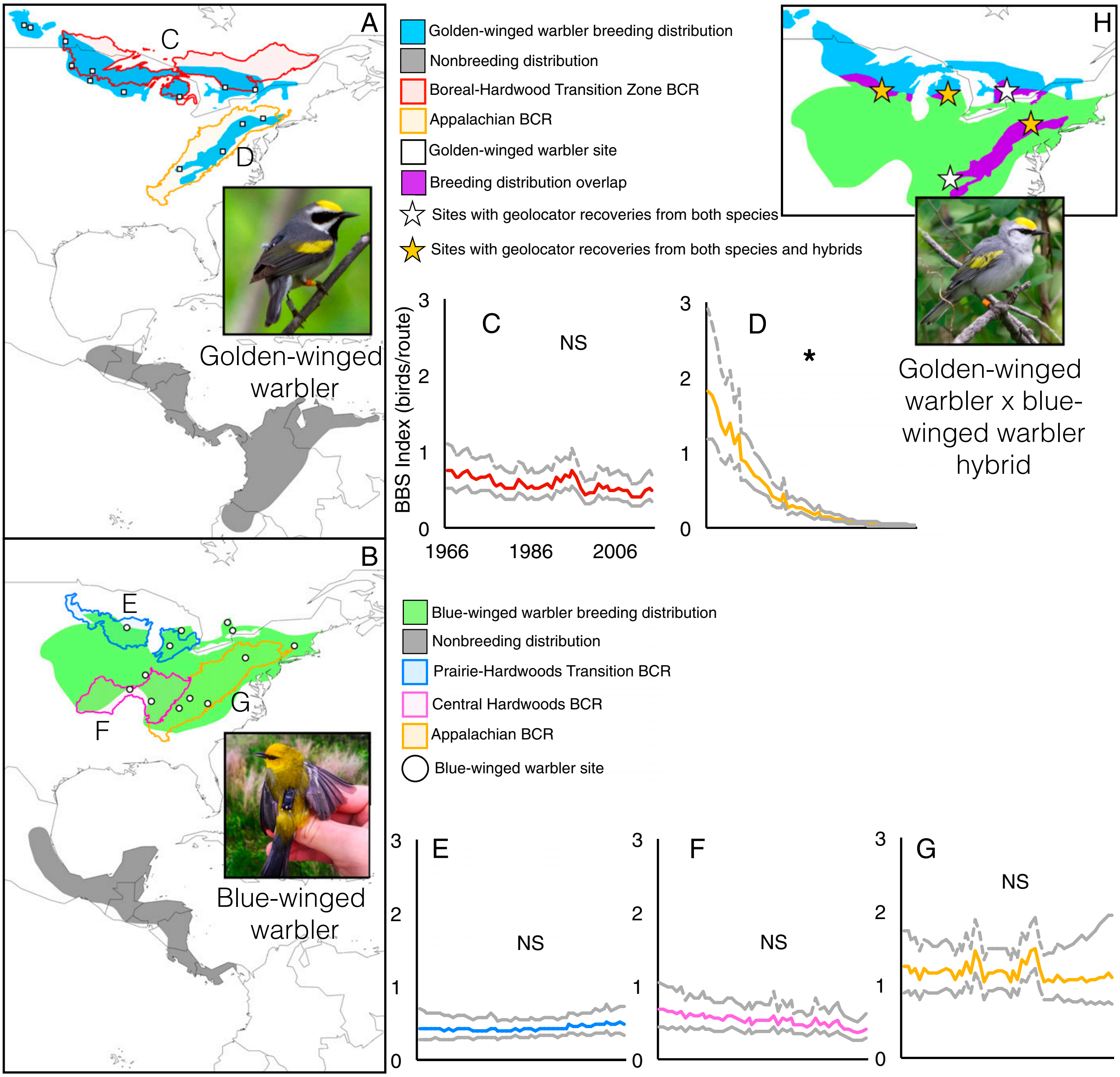

Fig. 1. Breeding and nonbreeding distributions of golden-winged warblers (*A*) and blue-winged warblers (*B*) with relevant BCRs outlined and study sites identified (keys are provided for details). Historical population trends of each species are provided for each relevant BCR from 1966 to 2015 [Breeding Bird Survey (44)]. In trend graphs, solid-colored lines represent annual indices of golden-winged warblers (*C* and *D*) and blue-winged warblers (*E*–*G*) (44) derived from hierarchical model analysis. Dashed gray lines represent 95% credible intervals around annual indices. Populations are noted as exhibiting nonsignificant trends [i.e., numerically stable (NS)] or significant trends [increasing or declining (*)] depending on whether the 95% credible interval around the trend estimate (from 1966 to 2015) includes zero. Note that all trend graphs have the same axis scales. (*H*) Breeding distribution overlap between golden-winged warblers and blue-winged warblers is presented. Range map information is adapted from BirdLife International (45), and nonbreeding ranges are buffered by 100 km.

from one another during the nonbreeding period (one-way ANOVA: $F = 1.3$; df = 3, 20; $P = 0.3$; Fig. S3). However, individual blue-winged warblers that spent the nonbreeding period in Central America exhibited a pattern such that individuals from more easterly breeding longitudes tended to occur farther east during the nonbreeding period (Fig. S3). Conversely, regionally isolated breeding populations of golden-winged warblers maintained their separation during the nonbreeding period, with the Great Lakes population of golden-winged warblers occurring farther west during the nonbreeding period, on average, than Appalachian Mountains breeding populations ($F = 213.4$; df = 1, 39; $P < 0.0001$; Figs. S2 and S3). Unsurprisingly, this pattern remained when we considered the relationship between individual breeding longitude and nonbreeding longitude within and between populations of golden-winged warblers (Fig. S4).

We found strong evidence to support our hypothesis that population trends of *Vermivora* warblers were associated with the distribution and isolation of historically stable and declining populations during the nonbreeding period. Historically stable populations of golden-winged warblers from breeding sites in the

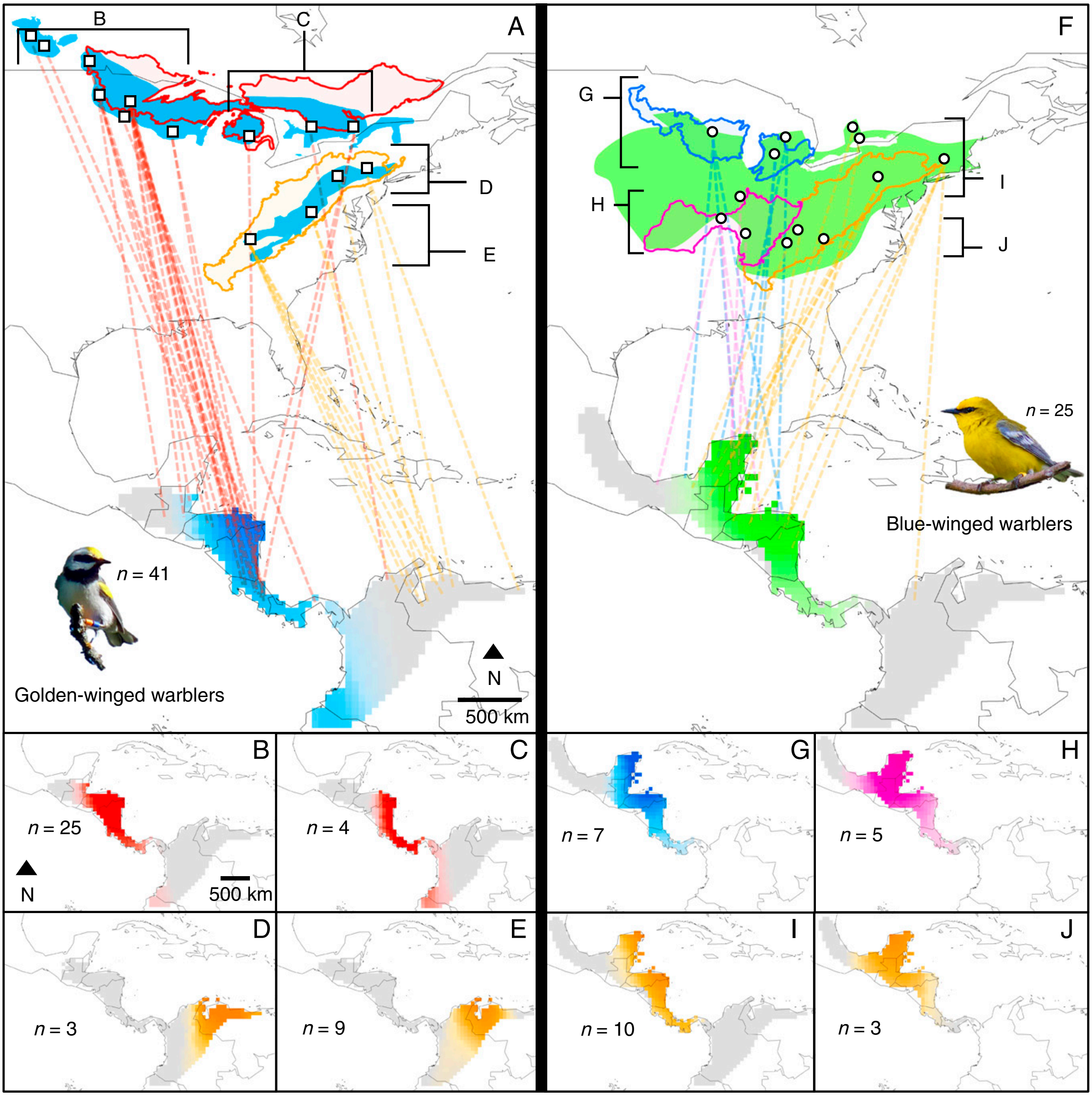

Fig. 2. Species-level average nonbreeding probability density function of golden-winged warblers (*A*) and blue-winged warblers (*F*). Dashed lines link individual warblers from breeding sites to their highest probability nonbreeding area. Colors correspond to BCRs in Fig. 1. Lines do not represent migration routes. Brackets define sites used to create population-level average probability density functions of golden-winged warblers (*B–E*) and blue-winged warblers (*G–J*). In all probability density functions, darker colors correspond to higher probability of use and the bottom 50% of probabilities are shown in gray to aid in visualization of core use areas. Range maps are adapted from BirdLife International (45). A description of BCRs is provided in Fig. 1.

Great Lakes region occurred throughout Central America during the nonbreeding period, whereas historically declining populations from breeding sites in the Appalachian Mountains region occurred in northern South America. Notably, blue-winged warblers from breeding sites in the Appalachian Mountains region (i.e., the same region in which golden-winged warblers are declining) occurred in Central America during the nonbreeding period alongside historically stable Great Lakes populations of golden-winged warblers. The remarkable similarities between these two species on the breeding grounds (e.g., nearly identical habitat use, phenology, life-history) and co-occurrence in geographical space suggest that the differential population trends observed between populations of golden-winged warblers and blue-winged warblers breeding in the Appalachian Mountains region are likely driven by factors outside of the breeding period.

Intraspecific migratory connectivity between populations of golden-winged warblers was strong (i.e., individuals from distinct breeding populations used different areas during the nonbreeding period), and we are not aware of any other report of similarly strong connectivity from a range-wide study of distinct breeding

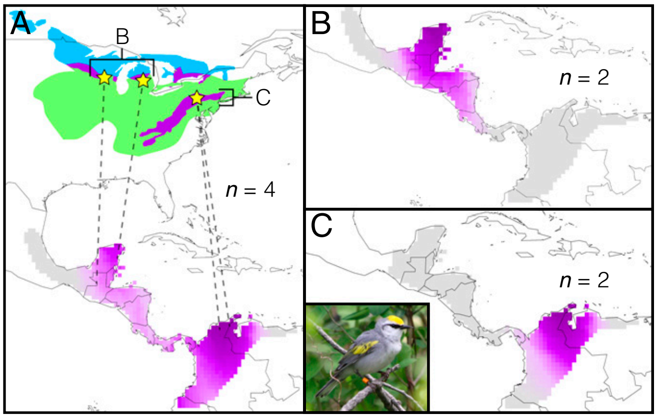

Fig. 3. Average nonbreeding probability density function of all phenotypic *Vermivora* hybrids (*n* = 4; *A*) and average nonbreeding probability density functions of hybrids from distinct breeding regions in the Great Lakes (*B*) and Appalachian Mountains (*C*) regions. Darker colors correspond to higher probability of use, and probabilities <50% of the maximum shown in gray to aid in visualization of core use areas. Lines represent links between individuals' breeding sites and areas of most probable nonbreeding sites but do not represent migration routes. Range maps are adapted from BirdLife International (45).

populations in another species of migratory songbird. Blue-winged warbler populations exhibited weak connectivity (i.e., individuals from distinct and isolated breeding areas co-occurred during the nonbreeding period) and occurred throughout Central America alongside golden-winged warblers from Great Lakes populations. We found weak connectivity (or high levels of dispersion) within Great Lakes golden-winged warblers and blue-winged warblers range-wide. For example, individual golden-winged warblers from breeding sites in Minnesota, where ~50% of the global population of golden-winged warblers breed (41), were dispersed throughout Central America, occurring from southern Mexico to Panama (maximum distance between two individuals was >1,500 km) during the nonbreeding period. Similarly, blue-winged warblers from breeding sites in Massachusetts were dispersed during the nonbreeding period from the Yucatan Peninsula, Mexico, to northern Colombia (maximum distance between two individuals was ~2,000 km). Without considering the individual that migrated to Colombia, Massachusetts blue-winged warblers were still broadly dispersed (maximum distance between two individuals was ~1,000 km). Conversely, populations of golden-winged warblers from breeding sites throughout the Appalachian Mountains were more concentrated in a relatively small area in northeastern Colombia and northwestern Venezuela during the nonbreeding period (maximum distance between two individuals from the same breeding site was ~600 km).

European migratory bird species that disperse broadly during the nonbreeding period are less likely to be declining than species with restricted distributions during the nonbreeding period compared with their breeding distributions (47, 48). We observed this trend at the population level, with greater nonbreeding dispersion among numerically stable populations (i.e., golden-winged warblers breeding in the Great Lakes and all populations of blue-winged warblers) compared with declining breeding populations (e.g., golden-winged warblers breeding in the Appalachian Mountains), suggesting that migratory diversity (i.e., within-population variation in migratory routes and/or destinations) may be linked to population trends at both species and population levels. Tracking additional individuals may uncover greater dispersion, but golden-winged warblers from Appalachian Mountains breeding populations were overrepresented in our sample based on estimated population size [29% of golden-winged warbler sample but only ~5% of the global population (41)]; therefore, we believe additional sampling will likely confirm low dispersion in this population.

The differential population trends observed in *Vermivora* warblers using Central American vs. South American nonbreeding areas may be caused by a variety of factors. The complexity of the ecological relationships and resource requirements that exist throughout the annual cycle of a migratory species makes it unlikely that there is a single driver of these trends. However, the identification of spatial isolation between these populations of *Vermivora* warblers suggests that the drivers of historical declines in populations of Appalachian Mountains-breeding golden-winged warblers are linked to their nonbreeding distribution in northern South America or the migratory pathways they use between breeding and nonbreeding locations. The cause of the precipitous declines of golden-winged warblers that use this region during the nonbreeding period need not be tied to a single factor, but may be the result of a reliance on a region that has experienced extensive anthropogenic exploitation and changes in land use over the past century (49–52).

Aerial photographs and/or satellite imagery covering the entire nonbreeding distribution of *Vermivora* are not available for the period when Appalachian Mountains populations of golden-winged warblers experienced their steepest declines [~1970–1980 (44)]. Furthermore, it is unlikely that golden-winged warblers in the Appalachian Mountains region began declining in 1966, the first year of the Breeding Bird Survey (41). Therefore, clearly identifying a mechanism related to these declines is challenging. Using modeled estimates of historical land-use and land-cover change (HYDE 3.1) (53–55) (*SI Materials and Methods*, in which a detailed description of the analysis is provided, and Fig. S5) for the region, we found that forest-dominated landscapes at appropriate elevations for golden-winged warblers [i.e., 200–2,400 m above sea level (41)] were converted to other, nonforest land uses in northern South America at a disproportionate rate compared with Central America (53–55) (Fig. 4). The loss of the forest-dominated landscape within the appropriate elevation envelope for golden-winged warblers in northern South America began in the early- to mid-1940s and continued through 1980 (Fig. 4). Based on our description of the migratory connectivity of *Vermivora*, these dramatic regional shifts in land use would likely affect primarily golden-winged warblers from Appalachian Mountains breeding populations (Fig. 2). Simple linear regressions reveal that these predicted changes in the amount of forest-dominated land in population-specific nonbreeding ranges explain significant variation in the breeding population trends of all three groups [(*i*) (blue-winged warblers range-wide, 1966–2010: $F = 16.3$; df = 1, 8; $P = 0.004$; (*ii*) Great Lakes populations of golden-winged warblers, 1966–2010: $F = 13.5$; df = 1, 8; $P = 0.006$; and (*iii*) Appalachian Mountains populations of golden-winged warblers, 1966–1990: $F = 77.7$; df = 1, 4; $P = 0.0009$] (Fig. 4). This evidence provides a parsimonious and temporally synchronized explanation for the observed differences in breeding population trends among these three groups of *Vermivora* warblers.

Certainly, the loss of appropriate humid mid-elevation tropical forest cover types caused by changes in land use, or other consequences of human activities, may have direct effects on the survival of nonbreeding golden-winged warblers (56). However, habitat fragmentation, reduced habitat quality, and habitat loss may have nonlethal effects that lead to lower reproductive success and survival of individuals that return to North America to breed (19, 57). Additionally, golden-winged warblers migrating to northern South America from northern Appalachian Mountains breeding sites travel >5,000 km farther than golden-winged warblers migrating between Central America and Great Lakes breeding sites each year (29). Therefore, northern Appalachian breeding populations of golden-winged warblers may be more sensitive to declines in available food resources, phenology mismatches during migration, and/or higher risk of encountering

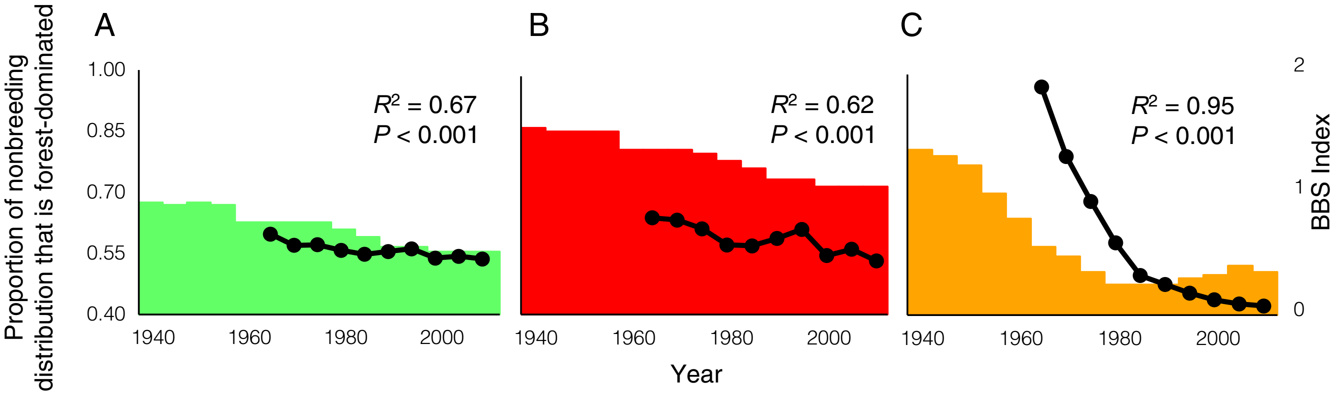

Fig. 4. Bar charts showing the modeled proportion of forest-dominated land cover at 200–2,400 m above sea level in the nonbreeding distribution of blue-winged warblers (*A*; Central America, southern Mexico), Great Lakes breeding populations of golden-winged warblers (*B*; Central America), and Appalachian Mountains breeding populations of golden-winged warblers (*C*; northern South America). Overlaid lines show breeding population trends (44) of each group over the same time scale. Axes for the proportion of forest (left *y* axis) and the Breeding Bird Survey (BBS) index (right *y* axis) are the same for all respective plots. Summary statistics of simple linear relationships of BBS index as a function of the proportion of forest-dominated land cover are presented. Regressions in blue-winged warblers (*A*) and Great Lakes breeding populations of golden-winged warblers (*B*) use all available years. The regression for Appalachian Mountains breeding populations of golden-winged warblers used the period 1966 to 1990 during the steepest decline and before the population was effectively reduced to near zero. Land-use data are from the HYDE 3.1 database (53–55).

predators or other obstacles during migration (16, 17, 57–59). Conversely, *Vermivora* that occur in Central America during the nonbreeding period migrate shorter distances to breeding sites and are more widely dispersed during the nonbreeding period such that the effects of potentially limiting factors are not likely to be experienced by all individuals of a population. A future productive research focus may be identifying potential limiting factors during migration so as to assess the impact of differential migration strategies on population trends of *Vermivora* warblers.

We did not observe intermediate nonbreeding site affinity in probable first-generation hybrids [identified based on plumage characteristics (60)]. Hybrids with intermediate migratory traits have been described in other species (61), but the hybrids we monitored did not occur in areas that we could differentiate from one of the parental types (e.g., hybrids from the Appalachian Mountains did not occur in Panama). However, if hybrids and parental types used nonbreeding areas separated by short distances (i.e., <250 km), we would be unlikely to differentiate those sites due to the spatial resolution of geolocator data. Future research with higher resolution technology (i.e., satellite-enabled markers, global positioning system-enabled markers) may be useful in determining if hybrids exhibit intermediate phenotypes in nonbreeding site affinity that are unable to be identified with geolocators. It is challenging to identify meaningful patterns in the nonbreeding distribution of two hybrids captured in the Great Lakes portion of the breeding distribution as there is little differentiation in the nonbreeding ranges of blue-winged warblers and golden-winged warblers breeding in that region. However, the two phenotypic hybrids we tracked from the Appalachian Mountains wintered in northern South America, where golden-winged warblers from the Appalachian Mountains occur during the nonbreeding period. We note, however, that our sample of hybrids may be influenced by ascertainment bias in that we only captured and attached geolocators to hybrids that returned to breeding areas after successfully migrating to and from nonbreeding locations, reducing any opportunity to identify migration to poor-quality or inappropriate nonbreeding locations that negatively influenced survival or breeding propensity (13). It is likely that the hybrid individuals we captured and tracked originated from interspecific pairings or extrapair copulations between parental species in the Appalachian Mountains region. Given the results of this study, the hybrid offspring of these pairings likely received genetic information from a blue-winged warbler that wintered in Central America and a golden-winged warbler that wintered in northern South America. If a subset of hybrid individuals produced in the Appalachian Mountains with a genetic predisposition to migrate to Central American nonbreeding areas does not survive (possibly due to a multitude of reasons, including a recombination of alleles that results in maladaptive migratory orientation or another postzygotic barrier), disperses to breeding areas that are outside the Appalachian Mountains (i.e., nonbreeding site affinity and breeding site affinity are genetically linked), or does not exhibit breeding behavior, our sample of hybrids would likely be biased. Further research is required to fully account for the behaviors of hybrid *Vermivora* warblers during their first migration and winter and to identify specific genomic regions associated with different migratory phenotypes (62).

It is unlikely that golden-winged warblers are the only broadly dispersed Nearctic-Neotropical migrant songbird species that exhibits strong migratory connectivity and shows population trends consistent with being limited by nonbreeding factors. Our results confirm that migratory connectivity and the nonbreeding distribution of species can be linked to breeding population trends. However, range-wide geolocator studies are expensive and logistically challenging, and being able to identify potential candidate species that may exhibit these similar relationships without mounting a continent-wide study would be valuable. We therefore identified 25 species of Nearctic-Neotropical migrant passerines that have similar nonbreeding distributions to *Vermivora* warblers and broadly dispersed breeding distributions (*Materials and Methods*, Fig. S6, and Table S2). Of those 25 species, we identified 16 (64%) that showed structured differences in regional breeding population trajectories that could be caused by nonbreeding factors (Table S2). Six of those species showed similar patterns in range-wide population trends as golden-winged warblers (declines from 1966 to 1990, followed by stable trends from ∼1990 to 2015). Without detailed knowledge of the factors affecting population trends of these species, we predict their regionally distinct breeding population trends may be linked to migratory connectivity and nonbreeding distribution similar to what we observed in golden-winged warblers. The Kentucky warbler (*Geothlypis formosa*) stands out as one of the most likely candidates to exhibit strong migratory connectivity based on these assumptions (Fig. S6 and Table S2). Kentucky warblers exhibit a dichotomous north-south pattern in their breeding population trends and occur in the same regions as golden-winged warblers during the nonbreeding period. In addition, range-wide declines starting in 1966 have leveled off and global population trends have been stable since ∼1990, similar to golden-winged warblers. Kentucky warblers may be another species that exhibits strong migratory connectivity, and like golden-winged warblers, signals of strong connectivity (e.g., coinciding population declines) may be shared among species that occupy similar regions and are affected by similar limiting factors during the annual cycle.

More complex scenarios may explain the patterns in population trends exhibited by Kentucky warblers and the other species we have promoted as potential candidates to exhibit strong migratory connectivity. There also are factors (e.g., regionally specific breeding-ground factors) that could obfuscate or mask the purported signals that we used to identify species that may exhibit strong migratory connectivity. Identifying species with strong connectivity and understanding the implications of such strong connectivity are timely and important (63). If anthropogenic changes in land-use patterns in northern South America are identified as a key factor driving population declines of golden-winged warblers occurring in that region during the nonbreeding period, then the strong migratory connectivity we observed in golden-winged warblers may constitute an evolutionary trap (64). In this case, anthropogenic factors that are limiting populations may be occurring over a relatively short period and outpacing the natural ability of these populations to adapt. Understanding the implications (65, 66) and diversity (67–69) of patterns in the distribution of migratory species throughout the annual cycle will aid in predicting the effects of continuously changing anthropogenic factors on migratory species globally (70).

Conclusion

We provide details on the distribution and migratory connectivity of two closely related, Nearctic-Neotropical migrant songbird species, which recent genetic evidence suggests may be plumage morphs of a single-species complex. More importantly, we show through a thorough sampling of populations throughout these species' breeding ranges that strong connectivity leading to the isolation and segregation of populations during the nonbreeding period may be associated with patterns in breeding population trends. Unlike blue-winged warblers, golden-winged warblers exhibited strong migratory connectivity, with declining populations occurring exclusively in northern South America. Identifying the factors that led to the severe declines and local extirpation of golden-winged warblers in the Appalachian Mountains region will be critical for their long-term conservation. Our observations of migratory connectivity in *Vermivora* warblers represent a major advance in understanding the unique drivers shaping migration strategies; the distribution of populations throughout the annual cycle; and, ultimately, the evolutionary trajectories of migrants in the rapidly changing Anthropocene. Studies like ours may benefit future research focused on identifying genomic regions associated with specific migratory behaviors. Our findings highlight the value of collecting information about the annual movements of species across their distribution, and studies like ours can aid in the conservation of migratory species, such as *Vermivora* warblers, in the future.

Materials and Methods

Study Area and General Procedures. We studied golden-winged warblers, blue-winged warblers, and their hybrids across their breeding distributions in eastern North America from 2013 to 2017. A small portion of these results include a reanalysis of geolocator data (https://conservancy.umn.edu/handle/11299/183086) reported by Kramer et al. (29). Study areas were chosen based upon location (i.e., representing the greatest geographic distribution), density of *Vermivora* warblers, and ease of access. *Vermivora* warblers are diverse-forest species that are often associated with young, regenerating forest surrounded by a larger matrix of mature forest, and our study sites reflected the full range of land-cover types used by these species (71). We captured all warblers in mist nets using conspecific call and song broadcasts. Upon initial capture, we determined age and sex, assigned a phenotypic species (i.e., typical golden-winged warbler, typical blue-winged warbler, hybrid) based on plumage traits (60), and weighed and banded each individual with a US Geological Survey/Canadian Wildlife Service aluminum band and one to three plastic color leg bands to aid in future identification. We attached geolocators (model ML6240, 2-min light-sampling regime; Biotrack Ltd.) to male *Vermivora* warblers that we determined to exhibit territorial or breeding behaviors (i.e., resident, nonmigratory behaviors) using an adapted leg-loop harness that was developed and tested on this species complex without any known negative effects (72–74). All birds were released after processing, and we monitored geolocator-marked birds for signs of stress during a brief (∼1–15 min) acclimation period.

The year following deployment, we returned to the sites where we marked warblers with geolocators and attempted to recapture all marked birds that returned. *Vermivora* warblers exhibit high site fidelity when their breeding habitat is not altered or removed and often return to the same territories year after year (73, 75). Thus, we began searches for returning geolocator-marked birds at the site where they were marked the previous year. We expanded our search radius to include appropriate breeding cover types within ∼1–3 km of the deployment site depending on the site and the surrounding landscape. We used the same methods as described above to lure returning geolocator-marked individuals into mist nets. We then removed the geolocator and released the warbler at its capture location. We attached new geolocators to a subset of individuals that successfully carried a geolocator in 2013 or 2014 in an attempt to understand whether individuals use different nonbreeding areas in different years (a discussion of how we treated individuals with multiple years of data is provided in the next section).

We captured and marked birds following Protocol 561, approved by the University of Tennessee Institutional Animal Care and Use Committee; Protocol 104A80575, approved by the University of Minnesota Institutional Animal Care and Use Committee; Virginia Commonwealth University Institutional Animal Care and Use Committee Proposal 10230; and Protocols 07-0303 and 10-0201 approved by the West Virginia University Institutional Animal Care and Use Committee.

Geolocator Data Analysis. We downloaded and decompressed raw light-level data from geolocators using BASTrack software (Bastrack Ltd.). All additional processing and analyses were conducted in R (76). Briefly, we followed the methods described by Kramer et al. (29) to derive nonbreeding probability density functions for each individual during the tropical dry season (i.e., January 1–February 28). This period represents the nonbreeding period when we assumed individuals were residing on nonbreeding territories and were exposed to the least environmental shading. We used FLightR (77) to estimate spatially explicit likelihood surfaces (∼0.5° cell size) for each transition period (i.e., sunrise, sunset) from January 1 to February 28. We used a subset of data or data from a different portion of the nonbreeding period if data were unavailable from January 1 to February 28 (due to geolocator failure or occlusion of the light sensor; Table S1). We then averaged all transition-derived likelihood surfaces for each individual to produce an average probability density function showing the most probable regions used by that individual during the nonbreeding period. We transformed individual nonbreeding likelihood surfaces into probability density functions by dividing each likelihood surface by the sum of its surface. We averaged nonbreeding probability density functions of individuals from the same breeding populations to achieve a population-level probability density function representing areas most likely used by an individual warbler from each population during the nonbreeding period.

Because that portion of the Neotropics used during the nonbreeding period by *Vermivora* warblers extends primarily from west to east, we used longitude as a proxy for individual nonbreeding site location in our calculations and estimations of overlap. Longitude is more accurate than latitude in geolocator analyses and can be useful for determining movement and location (78), especially in terrestrial organisms that are constrained to land during the period of interest but travel through regions that are bounded to the north and south by expansive water (e.g., Central America). To estimate nonbreeding site longitude, we extracted the longitude of the highest probability cell in the probability density function for each individual. We averaged the nonbreeding probability density functions for warblers for which we collected 2 y of data (n = 6) so that those individuals did not bias estimates of average nonbreeding distribution of populations.

Statistical Analysis. We evaluated differences between the nonbreeding distributions of populations using linear regression and one-way ANOVA and post hoc Tukey honest significance difference tests in R unless otherwise noted. Results of all tests were considered significant at $\alpha = 0.05$. We investigated the level of migratory connectivity in populations of *Vermivora* warblers based on BCRs for which annual population-level sampling is conducted and reported by the Breeding Bird Survey (44). Golden-winged warbler populations are largely contained within two BCRs: Boreal Hardwood Transition BCR (S12; i.e., Great Lakes population) and Appalachian Mountains BCR (S28; i.e., Appalachian Mountains population). Blue-winged warblers are more widespread, but our study sites fell primarily in three BCRs: Prairie Hardwood Transition BCR (S23), Central Hardwoods BCR (S24), and Appalachian Mountains BCR (S28). For both blue-winged warblers and golden-winged warblers, we split the Appalachian Mountains BCR into northern and southern halves (separated at ∼39° N) to investigate potential differences in the nonbreeding distributions of these groups of *Vermivora*. For golden-winged warblers, we also split the Great Lakes population (BCR 12) into eastern and western portions (∼85° W) for the same purpose.

Predicting Other Species with Nonbreeding Population Structure. We used range data from BirdLife International (45) to visually inspect the nonbreeding distributions of Nearctic-Neotropical migrant songbird species. We identified species that occurred in both Central America and South America during the nonbreeding period, as observed in golden-winged warblers (Table S2). We then visually inspected breeding period trend maps from the Breeding Bird Survey (44) and identified species that showed structured, regional variation in population trends. Finally, we visually inspected the survey-wide population trends for those species and noted their general population trends from 1966 to 1990 and 1991 to 2015. From 1966 to ∼1985, the range-wide population trend of golden-winged warblers declined as a result of the loss of individuals from the Appalachian Mountains breeding population. Following that decline, the range-wide population trend stabilized between 1985 and 1990. If other species had strong migratory connectivity similar to that which we observed in golden-winged warblers (i.e., isolated breeding populations occurring separately in Central America and South America during the nonbreeding period), one might expect to see a similar trend over the same period if limiting factors (e.g., deforestation, fragmentation; Fig. 4) were also affecting other Nearctic-Neotropical migrant populations. For example, yellow-throated vireos (*Vireo flavifrons*) are broadly dispersed throughout eastern North America during the breeding period and occur in both Central America and northern South America during the nonbreeding period. Eastern populations of yellow-throated vireos tend to be declining more than western populations. However, survey-wide trends of yellow-throated vireos revealed a stationary trend from 1966 to 1985, switching to increasing population trends from 1986 to 2010 (44). This suggests that the factors limiting yellow-throated vireos are different from those limiting golden-winged warblers; therefore, we predict that it is unlikely that yellow-throated vireos have similar nonbreeding population distribution and migratory connectivity as golden-winged warblers.

Data Accessibility

Data used for this study are freely and publicly available [data conservation provided by the Data Repository for the University of Minnesota (https://conservancy.umn.edu/handle/11299/193202)]. All other data used in this study were retrieved from publicly accessible databases.

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Attachment 22

Habitat availability may not be limiting potential Golden-winged Warbler *Vermivora chrysoptera* populations in the Unicoi Mountain Range of western North Carolina

Habitat availability may not be limiting potential Golden-winged Warbler *Vermivora chrysoptera* populations in the Unicoi Mountain Range of western North Carolina

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Introduction

The Golden-winged Warbler *Vermivora chrysoptera* breeds from the Upper Midwest (including areas of Canada) to the Southern Appalachians. The Upper Midwest hosts 95 percent of the entire species' population, while the remaining 5% breeds along the Appalachian Mountains, where the species is experiencing a decades-long population decline and range contraction (Rosenburg et al. 2017). Population declines are attributed to permanent land use conversion of breeding and wintering habitat, maturation of early seral breeding habitat, and climate change (Roth 2012, Rohrbaugh et al. 2017). Early seral breeding habitat is specifically needed in small patches for the actual nest sites. These patches are embedded within territories and home ranges that also include mature closed-canopy forest habitat (Rohrbaugh et al. 2017).

At the southernmost extent of the range, the interagency Golden-winged Warbler Working Group (GWWG) recognizes four focal areas in western North Carolina, including the Unicoi Mountains (Focal Area A18). The estimated population for focal area A18 in 2010 was 300 individuals using an estimated 1500 acres of breeding habitat (Roth et al. 2012). Based on an assumption that the population is limited by suitable habitat availability, the GWWG has established a goal of raising the population to 600 individuals by 2050 through the creation of 1500 more acres of available early seral nesting habitat throughout focal area A18 (Roth et al. 2012).

The Unicoi Mountains include six areas of high wildland value identified for potential wilderness recommendation (Unicoi Mountains proper, Upper Bald River Wilderness Study area, Snowbird Creek, Joyce Kilmer Slickrock extensions, and Santeetlah Bluffs). These areas, known as Mountain Treasures, were first prioritized for wilderness management in 1992 based on their relatively large contiguous blocks of mature, closed-canopy forest scoring low on measures of human impact (McClure 1992). The landscape is also interspersed with areas of early successional habitat around and within relatively small patches of regenerating timber harvests conducted within the past twenty years, overgrown wildlife openings, and grassy balds.

We conducted three seasons of fieldwork to determine the presence, absence and breeding status of the GWWA in the Unicoi Mountains landscape by surveying these different types of the available early successional habitat embedded within this mature forest landscape. While these areas surveyed were not necessarily model GWWA habitat, we surveyed the range of early successional habitat available across this landscape at that time. We also surveyed for Chesnut-sided Warbler *Dendroica pensylvanica*, a species that uses similar habitat (Collins et al. 1982, Richardson and Brauning 2013) that can result in direct interspecific competition (Jones 2015). We contend that a better understanding of GWWA status and use of the existing available habitat will help guide the management decisions undertaken to increase GWWA habitat.

Management for GWWA habitat can be incorporated into commercial timber harvests or undertaken more directly and intentionally through the maintenance of existing wildlife openings, the creation of new wildlife openings in existing regenerating stands, and through refined management prescriptions for the ongoing maintenance of grassy balds. We proposed at the outset that it would be hard to justify creation of GWWA habitat in mature closed canopy stands at the expense of the conservation values inherent in these stands if existing early successional habitat is not fully occupied regardless of whether the habitat is ideal GWWA habitat as it could be improved upon. We also proposed at the outset that if early successional habitat were by and large unoccupied by the GWWA, that it would be prudent to undertake non-commercial management in these areas in order to create better GWWA breeding habitat before designing harvests in mature closed canopy forest using GWWA breeding habitat creation as the justification.

Methods

During the 2014 field season we followed the survey protocol in use by the Golden-winged Warbler Working Group in western North Carolina. This protocol employed a ten minute passive point count and a period of playback of the male Golden-winged Warbler Type 2 song. The surveys of 2014 were conducted in the middle of June within the recommended timeframe of the protocol employed that year by the southern Appalachian members of the GWWG.

During the 2015 and 2016 field seasons we switched to an updated protocol in use by the GWWG in western North Carolina. This protocol required a three minute passive point count, playback of male Golden-winged Warbler Type 1 and Type 2 song, and playback of the Black-capped Chickadee mobbing call. In accordance with survey protocols, we located multiple survey points within each site such that we incorporated shrub and forest habitat edge and some interior within the range of auditory detectability for the point counts. All survey points were separated by 250 meters to avoid double registration of singing males. The survey timeframe was adjusted by the GWWG to occur in the middle of May for 2015 and 2016 and we adjusted our times accordingly.

The passive point count of the surveys for all three field seasons included noting the presence and absence of the Chestnut-sided Warbler *Dendroica pensylvanica*. which also breeds in early seral forest habitat (Collins et al. 1982, DeGraaf and Rappole 1995, DeGraaf and Yamasaki

2001, the Golden-winged Warbler Conservation Plan and Review 2012, Richardson and Brauning 2013, Jones 2015, North Carolina State Wildlife Action Plan 2015).

We conducted the point count surveys during the breeding seasons of 2014, 2015, and 2016 in several of the Unicoi Mountains Mountain Treasure areas. The Unicoi Mountains include portions of the Great Smoky Mountains National Park and approximately 400,000 acres of land in the Cherokee and Nantahala National Forests in eastern Tennessee and western North Carolina. The topography varies from lower elevation valleys and bottomlands around the Little Tennessee River and impounded tributaries at approximately 2000 feet to over 5,000 feet across the balds in the vicinity of the Cherohala Skyway. Cover includes mesic hardwood bottomlands across a pronounced mountain gradient including cove hardwood, mesic to dry-mesic oak, and xeric oak and pine, through high elevation red oak, to northern hardwood and intermittent grassy balds.

The balds surveyed included Bob Stratton Bald, Huckleberry and Little Huckleberry Balds, Whigg Meadow, and Hooper Bald (Figure 2). The balds are actively maintained by the United States Forest Service as areas of grass sometimes interspersed with patches of shrub cover. We conducted lower elevation surveys below the balds in the Santeetlah Bluffs Mountain Treasure and other Mountain Treasure areas adjacent to the Joyce Kilmer-Slickrock Wilderness and Wilderness Extensions. These surveys were conducted within maintained wildlife openings, other areas maintained in grass and shrub cover such as Swann Meadow, and in areas of regenerating forest harvested within approximately the past twenty years.

We also surveyed several points approximately 12 miles east of the Unicoi Mountains within Cheoah Bald Mountain Treasure in the Nantahala National Forest near Stecoah Gap (Figure 3), a point near Robbinsville, North Carolina (Figure 3) approximately 15 miles south and east of the western Unicoi, and points approximately 28 miles south and slightly east near Franklin, North Carolina (Figure 4). Golden-winged warblers were known to have been sighted in some of these locations in previous years, leading us to include these sites in our surveys as these might be a source of the nearest potential dispersing individuals. The Stecoah Gap sites included a wildlife opening and several points within a regenerating shelterwood harvested approximately 10 years earlier. The sites near Robbinsville were located within and adjacent to maintained powerline rights of way. The sites near Franklin were along road rights of way parallel to wetland and riparian buffers and meadows. We also conducted surveys further east and south within the Unicoi Mountains to identify potential nearest source populations at lower elevations where the species had been previously sighted.

Results

No Golden-winged Warblers were observed on any of the balds over the 2014, 2015, and 2016 field seasons (Table 1). During the 2014 field season, Chestnut-sided Warblers (CSWA) were observed on each of the six points on Bob Stratton Bald (Table 1). In 2015, Chestnut-sided

Warblers were observed on two of the points on Bob Stratton Bald, at one of the Whigg Meadow points, and at two of the Hooper Bald points, and in 2016, they were observed on two of the points on Bob Stratton Bald, on four of the points on Big and Little Huckleberry Balds, and on one of the Hooper Bald points (Table 1).

No Golden-winged Warblers were observed at the middle to lower elevation early seral forest patches below the balds in the Santeetlah Bluffs Mountain Treasure in the 2015 and 2016 field seasons (Table 2). There were no surveys done at these sites during the 2014 field season (Table 2). Chestnut-sided Warblers were observed at several locations in early seral habitat (Table 2). In 2015, they were observed at one of the Johns Knob points and at one of the Swann Meadow points (Table 2), and in 2016 they were observed at the two Wildlife Opening points and one of the Swann Meadow Points (Table 2).

Golden-winged Warbler were observed in all three field seasons (Table 3) at several points east and southeast of the Unicoi Mountains. A male was observed on several sites near Stecoah Gap including a male in Stecoah Gap 1 in a wildlife opening in 2014 and 2015, and a male and a female at Stecoah Gap 1 in 2016 (Table 3). A Golden-winged Warbler was observed at the Murphy Road Point number 1 in each of the two years (2015 and 2016) that it was surveyed (Table 3), and one was observed at the Powerline number 1 point in the only year (2016) surveyed. No Chestnut-sided Warblers were observed at any of these points east and southeast of the Unicoi Mountains.

Discussion

Our fieldwork conducted over three breeding seasons found no GWWA on the sites we surveyed across the range of available early successional habitat in the western Unicoi Mountains. While this habitat is not necessarily the optimal habitat required by this species, our first objective, simply to determine the presence and breeding status in the Unicoi on the best potential habitat available was achieved. The presence of CSWA at many of these sites suggests that there was the potential for GWWA to breed on these sites. In spite of some species specific habitat selection differences, these two species are well known to breed in similar early successional habitat and sometimes the same sites. (Collins et al. 1982, DeGraaf and Rappole 1995, DeGraaf and Yamasaki 2001, the Golden-winged Warbler Conservation Plan and Review 2012, Richardson and Brauning 2013, Jones 2015, North Carolina State Wildlife Action Plan 2015) with breeding site selection resulting in direct competitive interactions (Jones 2015). These results suggest to us the need for a precautionary approach to where and how the creation of more GWWA habitat is undertaken.

There is ample unoccupied early successional habitat that though not necessarily ideal for GWWA, could be managed to create better GWWA habitat. Intentional GWWA management of these regenerating harvest sites, older wildlife openings, and grassy balds might be more effective than trying to create GWWA habitat in the context of commercial timber harvests in mature closed canopy forest. It would be an opportunity to learn more about the potential recruitment of GWWA to this area and establishment of a breeding population without sacrificing landscape-wide, under-represented mature closed canopy forest of the Unicoi Mountain Treasure areas.

We did find a GWWA male on territory in a wildlife opening in the Stecoah Mountains and several males nearby in a regenerating shelterwood harvest. We also visited sites with known GWWA near Robbinsville and Franklin within vegetation maintained for a powerline right of way and road right of way respectively. The habitat structure and composition of these sites with the GWWA present were comparable to the sites we surveyed in the western Unicoi based on qualitative inspection. The qualitative inspection is certainly no substitute for a quantitative vegetation sampling protocol. What is striking here is that of the four sites where GWWA were observed, three of them were created through intentional vegetation management outside of a commercial timber harvest (wildlife opening, powerline right of way, and road right of way.)

Another explanation for the existence of unfilled breeding habitat in region A18 is related to population declines driven by differential conditions on the nonbreeding grounds. The Great Lakes population winters in Central America, and the Appalachian population winters in South America (Hobson et al 2017). Kramer et al. (2018) confirmed differential migratory connectivity. They suggest that deforestation on South American wintering grounds over the last 80 years has preceded and been more significant than in Central America and might be responsible for the decline of the Appalachian breeding population. They further suggest that the Appalachian population has an overall longer distance migratory route thereby exposing them to extended periods of exposure to the hazards of long-distance migration, including a phenological mismatch to prey and local weather conditions at a stopover or breeding site.

Nonetheless, regardless of the contribution of events on the wintering grounds or on migration to Southern Appalachian GWWA population declines, we propose a low risk precautionary approach to the creation of more breeding habitat in region A18. Unoccupied early successional habitat in the Unicoi Mountain Treasures, though not necessarily ideal, does exist for the GWWA or could exist with intentional management of these areas undertaken for this species. In the interest of conserving the under-represented mature, close canopy forest and forest dependent bird species and other wildlife of the greater Southern Appalachians, we do not see compelling evidence to use creation of GWWA breeding habitat to justify harvest of mature closed canopy forest when other more cautious options exist.

Addendum: Golden-wing Warbler Presence and Absence in other work since 2016

Please note that since this work was completed in 2016, there have been additional surveys undertaken by other research teams that have documented GWWA presence in the Unicoi Mountains landscape. This is encouraging and can only add to our ability to make informed management decisions regarding the landscape-wide creation or improvement of GWWA breeding habitat in area A18.

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Table 1. Golden-winged Warbler (GWW) and Chestnut-sided Warbler (CSWA) bald surveys all years, Presence =P, Absence = A.

Point	**GWW 2014**	**CSWA 2014**	**GWW 2015**	**CSWA 2015**	**GWW 2016**	**CSWA 2016**
Bob Stratton bald 1	A	**P**	A	A	A	**P**
Bob Stratton bald 2	A	**P**	A	**P**	A	**P**
Bob Stratton bald 3	A	**P**	A	**P**	A	A
Bob Stratton bald 4	A	**P**	A	A	A	A
Bob Stratton bald 5	A	**P**	A	A	A	A
Bob Stratton bald 6	A	**P**	A	A	A	A
Little huckleberry bald 1	A	A	A	A	A	A
Little huckleberry bald 2	A	A	A	A	A	**P**
Big huckleberry bald 1	A	A	A	A	A	**P**
Big huckleberry bald 2	A	A	A	A	A	**P**
Huckleberry bald aprch 1	A	NA	A	A	A	**P**
Huckleberry bald aprch 2	A	NA	A	A	A	A
Whig meadow bald 1	A	A	A	**P**	A	**P**
Whig meadow bald 2	A	A	A	A	A	**P**
Whig meadow bald 3	A	A	A	A	A	**P**
Whig meadow bald 4	A	A	A	A	A	**P**
Hooper bald 1	A	NA	A	**P**	A	A
Hooper bald 2	A	NA	A	**P**	A	A
Hooper bald 3	A	NA	A	A	A	**P**

Table 2. 2015 Golden-winged Warbler (GWW) and Chestnut-sided Warbler (CSWA) lower elevation Unicoi Mountains surveys all years. Presence = P, Absence = A.

Point	**GWW 2015**	**CSWA 2015**	**GWW 2016**	**CSWA 2016**
Wildlife opening 1	A	A	A	P
Wildlife opening 2	A	A	A	P
Johns knob 1	A	P	NA	NA
Johns knob 2	A	A	NA	NA
Santeetlah Regeneration 1	A	P	A	A
Santeetlah regeneration 2	A	A	A	A
Santeetlah regeneration 3	A	A	A	A
Swann Meadow 1	A	A	A	P
Swann Meadow 2	A	P	A	
Swann Meadow 3	A	A	NA	NA
Fat gap 1	NA	NA	A	A
Fat gap 2	NA	NA	A	A
Fat gap 3	NA	NA	A	A

Table 3. 2016 Golden-winged Warbler (GWW) Chestnut-sided Warbler (CSWA) surveys east and southeast of the Unicoi Mountain surveys, Presence = P, Absence = A.

Point	**GWW 2014**	**CSWA 2014**	**GWW 2015**	**CSWA 2015**	**GWW 2016**	**CSWA 2016**
Stecoah gap 1	P	A	P	A	P (male and female)	A
Stecoah gap 2	A	A	A	A	A	A
Stecoah gap 3	A	A	A	A	P	A
Stecoah gap 4	A	A	A	A	A	A
Stecoah gap 5	NA	NA	A	A	A	A
Murphy road 1	NA	NA	P	A	P	A
Murphy road 2	NA	NA	NA	NA	A	A
Standing indian 1	NA	NA	NA	NA	A	A
Standing indian 2	NA	NA	NA	NA	A	A
Standing indian 3	NA	NA	NA	NA	A	A
Powerline 1	NA	NA	NA	NA	P	A

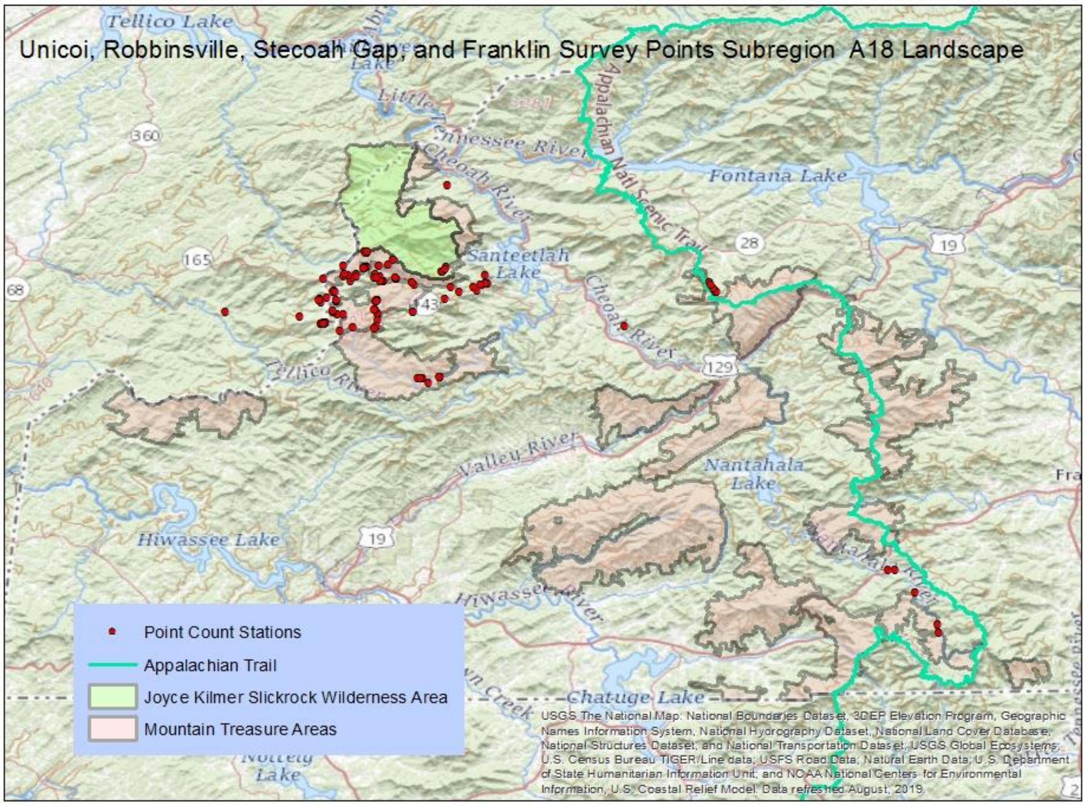
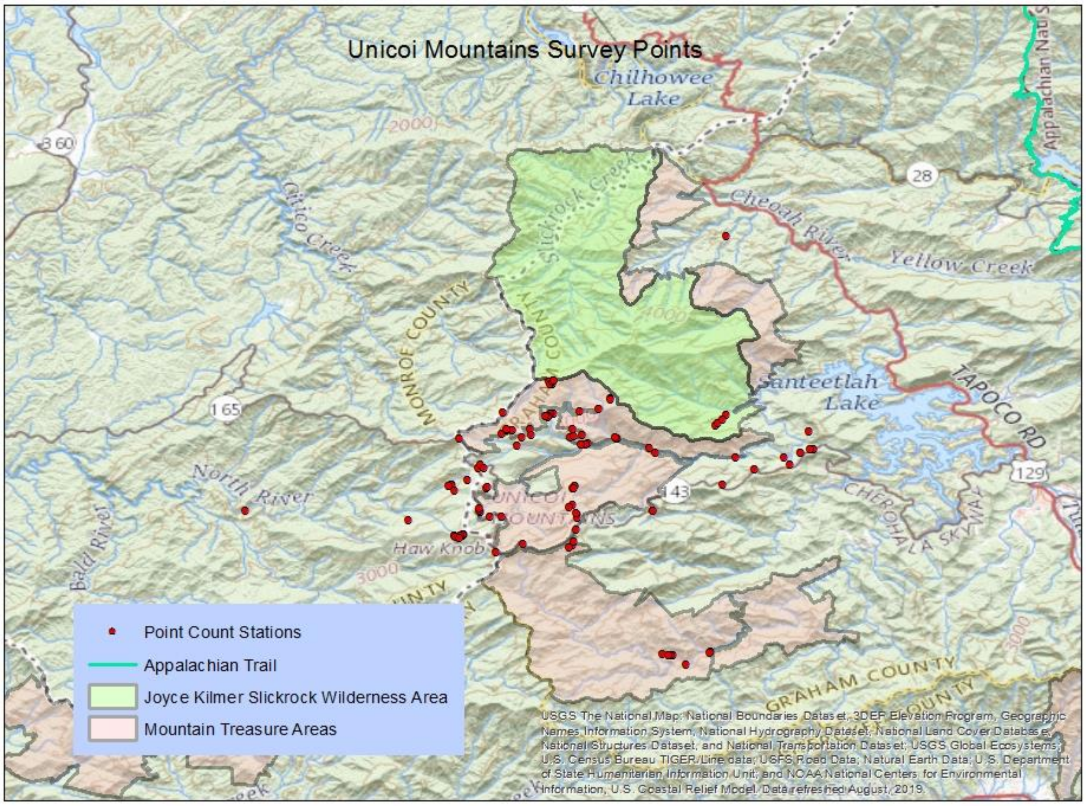
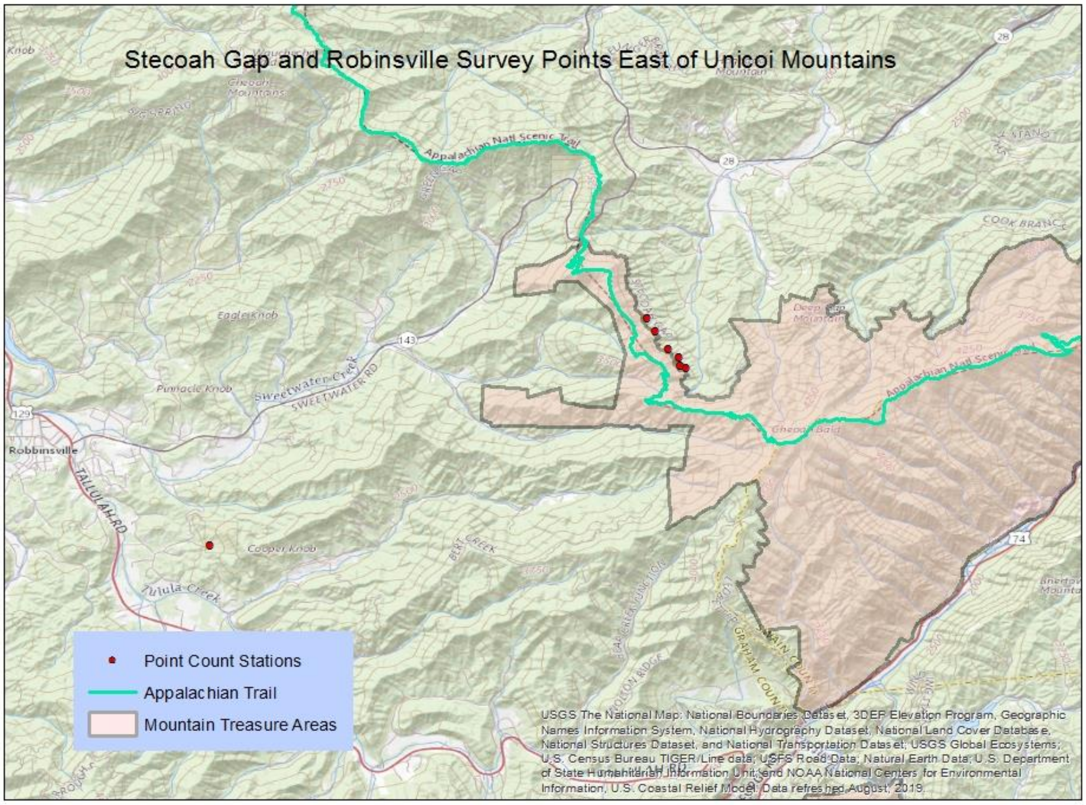


Figure 1. All survey points all years across Golden-winged Warbler Working Group Subregion A18.



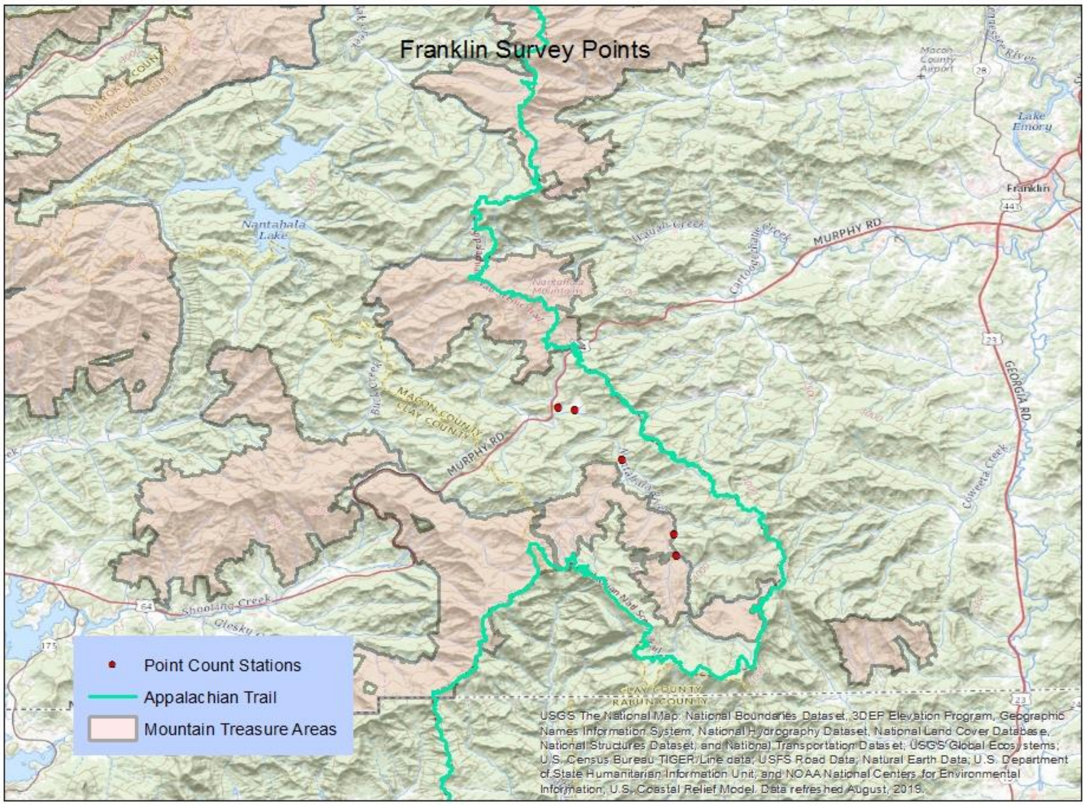
USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USFS Road Data; Natural Earth Data; U.S. Department of State Humanitarian Information Unit; and NOAA National Centers for Environmental Information, U.S. Coastal Relief Model. Data refreshed August, 2019.

Figure 2. Unicoi Mountains Survey Points in the Joyce Kilmer-Slickrock Wilderness, Mountain Balds and Lower Elevation Early Seral Habitat Patches within Unicoi Mountains Mountain Treasures.



USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USFS Road Data; Natural Earth Data; U.S. Department of State Humanitarian Information Unit; and NOAA National Centers for Environmental Information, U.S. Coastal Relief Model. Data refreshed August, 2019.

Figure 3. Survey Points East of the Unicoi Mountains in the Stecoah Gap Mountain Treasure and Robbinsville.



Franklin Survey Points

USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USFS Road Data; Natural Earth Data; U.S. Department of State Humanitarian Information Unit; and NOAA National Centers for Environmental Information, U.S. Coastal Relief Model. Data refreshed August, 2019.

Figure 4. Survey Points South of the Unicoi Mountains in Franklin, NC.

Attachment 23

Cerulean Warbler (*Dendroica cerulea*) Microhabitat and Landscape-level Habitat Characteristics in southernWest Virginia

The Auk 122(2):497–508, 2005

CERULEAN WARBLER (*DENDROICA CERULEA*) MICROHABITAT AND LANDSCAPE-LEVEL HABITAT CHARACTERISTICS IN SOUTHERN WEST VIRGINIA

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Abstract.—The Cerulean Warbler (*Dendroica cerulea*) is a species of conservation concern in eastern North America, where declines in its population have been documented over the past several decades. A high proportion of the population occurs in forested areas of southern West Virginia, where it may be threatened by loss and degradation of forested habitat from mountaintop mining and valley fill (MTMVF). We examined, from a landscape perspective, the effects of forest fragmentation (in particular, effects of fragment size and response to edges) on Cerulean Warblers, using territory mapping techniques and geographic information system (GIS) technology in portions of four counties in southwestern West Virginia. We quantified landscape characteristics from digitized aerial photographs and measured microhabitat characteristics on spot-mapping plots. Territory density of Cerulean Warblers was 4.6 territories per 10 ha in intact forest and 0.7 territories per 10 ha in fragmented forest. The best habitat model included both landscape and microhabitat variables and indicated that territory density increased with increasing snag density, percentage of canopy cover >6–12 m and >24 m in height, and distance from mine edge. Models for predicting microhabitat use at the territory level were weak, indicating that microhabitat characteristics of territories were similar to habitat available on spot-mapping plots. The species did not appear to avoid internal edges, such as natural canopy gaps and open-canopy or partially open-canopy roads. Territory placement on ridges was greater than expected, and in bottomlands (ravines) and midslopes less than expected, given availability. Fifty percent of all territories were on ridges. Preference for ridges suggests that MTMVF may have a greater effect on Cerulean Warbler populations than other sources of forest fragmentation, given that ridges are removed in MTMVF. Our data indicate that Cerulean Warblers are negatively affected by mountaintop mining from loss of forested habitat, particularly ridgetops, and from degradation of remaining forests, as evidenced by lower territory density in fragmented forests and lower territory density closer to mine edges. *Received 20 February 2003, accepted 1 November 2004.*

Key words: Cerulean Warbler, *Dendroica cerulea*, habitat, mining, territory density.

Características del Microhábitat y del Hábitat a Nivel de Paisaje de *Dendroica cerulea* en el Suroeste de Virginia

Resumen.—*Dendroica cerulea* es una especie con un estado de conservación preocupante en el este de Norteamérica, donde se ha documentado una disminución de sus poblaciones durante las últimas décadas. Una alta proporción de la población

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se encuentra en las áreas boscosas del suroeste de Virginia, donde las causas de la amenaza pueden estar relacionadas con la pérdida y degradación del hábitat boscoso debido a actividades mineras en las cimas de los cerros y el relleno de los valles. Desde una perspectiva a nivel del paisaje, examinamos los efectos de la fragmentación del bosque (en particular los efectos del tamaño de los fragmentos y las respuestas a los bordes) sobre *D. cerulea* utilizando técnicas de mapeo de territorios y tecnología de sistemas de información geográfica (SIG) en partes de cuatro condados del suroeste de Virginia. Cuantificamos las características del paisaje a partir de fotografías aéreas digitalizadas y medimos las características del microhábitat en los sitios en que se realizó el mapeo de los territorios. La densidad de territorios de *D. cerulea* fue de 4.6 territorios por 10 ha en bosques intactos y de 0.7 territorios por 10 ha en bosques fragmentados. El mejor modelo de hábitat incluyó tanto variables del microhábitat como del paisaje, e indicó que la densidad de territorios aumentó con la densidad de árboles vivos en pie, el porcentaje de cobertura del dosel entre 6–12 m y >24 m de altura y la distancia al borde de la mina. Los modelos para predecir el uso de hábitat a nivel de territorio fueron débiles, lo que indica que las variables de microhábitat de los territorios fueron similares al hábitat disponible en los sitios en que se realizó el mapeo de los territorios de las aves. Esta especie no pareció evitar los bordes internos, tales como aberturas naturales en el bosque, aberturas en el dosel o caminos con dosel semi-abierto. El establecimiento de territorios en las cimas fue mayor de lo esperado y en el fondo de las quebradas y laderas fue menor de lo esperado con relación a la disponibilidad. El 50% de todos los territorios estuvieron en las cimas. La preferencia por las cimas sugiere que las actividades mineras en las cimas de los cerros y el relleno de los valles pueden tener un mayor efecto sobre las poblaciones de *D. cerulea* que otras fuentes de fragmentación del bosque, dado que las cimas son removidas por las actividades mineras. Nuestros datos indican que las poblaciones de *D. cerulea* están siendo afectadas negativamente por las actividades mineras en la cima de los cerros debido a la pérdida de hábitat boscoso, particularmente en las cimas de las montañas, y por la degradación del bosque remanente. Esto se evidencia en una menor densidad de territorios en los bosques fragmentados y cerca de los bordes con las minas.

Cerulean Warblers (*Dendroica cerulea*) have been declining in many parts of their range (Sauer et al. 2004); a status assessment (Hamel 2000) suggested that the population is declining at "precipitous rates." The species has been identified by Partners in Flight as a priority for conservation in the upland forest community of the Ohio Hills and Northern Cumberland Plateau physiographic areas, which include southern West Virginia. Those two physiographic areas contain a large proportion of the total Cerulean Warbler population (Rosenberg et al. 2000). Southwestern West Virginia may represent a significant source population for this species in the eastern United States (Rosenberg and Wells 2000).

The primary threat to the species is loss and degradation of habitat (Hamel 2000, Hamel et al. 2004). In both the Ohio Hills and Northern Cumberland Plateau physiographic areas, a current potential risk to Cerulean Warbler populations is the coal-mining technique of mountaintop mining and valley fill (MTMVF). With this mining technique, rock and soil overburden is removed from an entire ridgetop to expose coal seams and is placed in head-of-hollow streams, creating valley fills. Once the coal is removed, reclamation includes returning some of the overburden to ridgelines, contouring valley fills, and seeding the site primarily to grasses and herbaceous vegetation. These extensive surface mines can affect areas on the order of 2,000 ha, converting a landscape that is predominantly forested to a landscape of predominantly early-successional habitats with remnant forest fragments (P. B. Wood et al. unpubl. data). It is imperative to understand how these landscape-level changes affect Cerulean Warblers, a species that inhabits large tracts of mature deciduous forest with large, tall trees. The species appears to use edges of small canopy gaps within large tracts; however, its use of openings and edges needs further study. High-priority research needs identified for Cerulean Warblers include occurrence and density in relation to landscape characteristics, especially in relation to forest fragmentation, habitat preferences in relation to vegetation structure, and response of populations to land management activities (Hamel 2000).

The major effects of MTMVF on Cerulean Warblers potentially include both loss and degradation of forested habitat. Large areas of mature hardwood forest are converted to early-successional habitat, resulting in outright loss of forested habitat. Remaining forest patches may be degraded because of fragmentation, area, and edge effects. Currently, there are no published studies documenting the effects of MTMVF on forest-dwelling songbirds as forests are lost and fragmented by mining activities. However, because of the large size of most MTMVF areas, it is possible that they have severe negative effects on populations of forest interior species, such as the Cerulean Warbler, that require large blocks of unfragmented forest for breeding. Duration of the habitat loss or fragmentation will depend on which post-mining land use is selected for an area. Nontimber post-mining land uses, such as grazing or development, will result in permanent fragmentation of forest habitats. Even with timber as the post-mining land use, it is estimated that many hundreds of years may be required for a functioning forest to regenerate on such sites.

The specific objectives of our study in southern West Virginia were (1) to compare Cerulean Warbler territory densities in forests fragmented by MTMVF mining with those in relatively intact blocks of forest, (2) to quantify landscape characteristics affecting Cerulean Warbler territory density, and (3) to quantify territory-level characteristics of Cerulean Warbler habitat.

METHODS

STUDY SITES

Our study sites were located in mature forest on and surrounding three MTMVF complexes within three watersheds in Boone, Logan, Kanawha, and Fayette counties, West Virginia (Fig. 1). One mine complex (2,003 ha), in Kanawha and Fayette counties, was in the

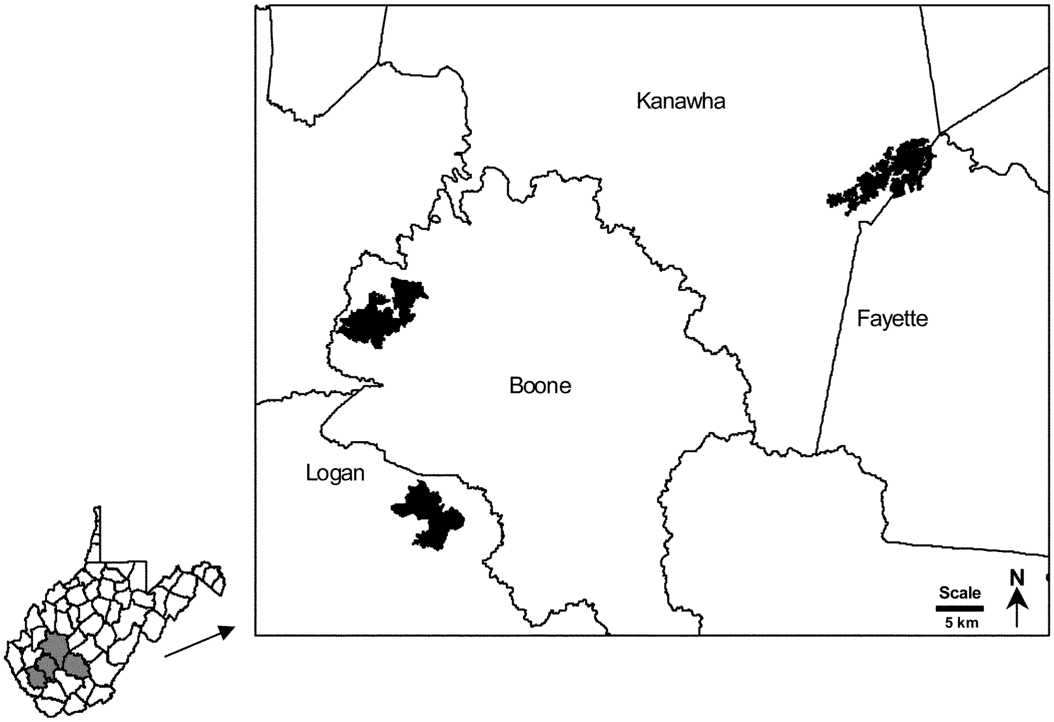

FIG. 1. Location of mountaintop mine and valley fill study sites in southern West Virginia.

Ohio Hills physiographic province; the other two (1,672 and 1,819 ha) were in the Northern Cumberland Plateau.

We established sampling plots within intact and fragmented forest sites. We defined fragmented forest sites as tracts of forest located within an MTMVF complex, surrounded primarily by reclaimed mine land. Generally they were long, narrow peninsulas of forest extending into reclaimed grassland habitat. We defined intact forest sites as extensive areas of unfragmented forest located near reclaimed MTMVF complexes, either within the same watershed as the mine complex or in an adjacent watershed (to minimize spatial variability). Although the sites consisted of relatively contiguous forest, they had some breaks in canopy cover from streams, roads, powerlines, and natural canopy gaps. We used some intact forest sites close to MTMVF areas to examine response to the large-scale edge associated with reclaimed mines.

The intact and fragmented forest areas are composed mostly of mature hardwood species, including oaks (*Quercus* spp.), hickories (*Carya* spp.), tulip poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), red maple (*Acer rubrum*), sugar maple (*A. saccharum*), and white ash (*Fraxinus americana*), with eastern hemlock (*Tsuga canadensis*) occurring in mixed conifer and deciduous areas. These stands are second-growth forests that appear to be approximately 60–80 years old, with evidence of periodic disturbances over the past several decades from firewood cutting, single tree harvesting, thinning, and understory forest fires.

TERRITORY MAPPING

In 2001, we established 6 intact forest plots (two within each watershed) and 19 plots in 15 fragments. We added two other intact plots in 2002.

Plots were placed near the center of 15 forest fragments ranging in size from 1 to 290 ha, which allowed us to examine territory density in relation to fragment size. In two larger fragments, two 10-ha plots were established, one near the center and one adjacent to a reclaimed grassland mine-edge to examine response to major edge type. In the largest fragment, three plots were established, one adjacent to edge (10 ha), one interior on a midslope (7.5 ha), and one along a stream (10 ha). For fragments of <10 ha, the whole fragment was surveyed for Cerulean Warblers; therefore, plot size was equal to fragment size.

All intact forest plots were 10 ha in size. Although intact forest plots were ≥100 m from the mine edge, they contained internal edges (e.g. roads, streams, and natural canopy gaps), giving us the opportunity to assess the effects of those edge types on Cerulean Warbler densities.

We surveyed Cerulean Warblers using spot-mapping (Bibby et. al 1992). Each fragmented forest and intact forest plot was surveyed at least 10 times from the first week of May to the first week of July each year (Bibby et al. 1992). Surveys were conducted from a half-hour after sunrise to 1030 hours EST. All surveys were conducted by 3–5 observers experienced in songbird identification and trained in territory-mapping procedures.

Territories were delineated and boundaries established using the minimum convex polygon method. Territory centers were defined as the intersection of the two longest axes bisecting the polygon and thus represented the approximate geographic center of the territory or the actual nest site when its location was known. For fragments with more than one plot, we calculated the average density of territories for the fragment. The maximum number of territories per 10 ha on each plot over the two years was used in analyses.

MICROHABITAT SAMPLING

We quantified microhabitat characteristics within each territory-mapping plot and at each Cerulean Warbler nest using modified methods from BBIRD (Martin et. al 1997) and James and Shugart (1970). We established two 0.04-ha vegetation quadrats per hectare in each territory-mapping plot. Quadrats were systematically distributed every ~100 m throughout the plot (Ratti and Garton 1994), except at sites that were used in an earlier study in 1999–2000 (P. B. Wood et al. unpubl. data). We used existing microhabitat information from those sites. Sampling methods were the same in both studies, and habitat conditions had not changed; we collected additional microhabitat measurements only if the sample size was <2 quadrats per hectare. After territories were delineated, one 0.04-ha quadrat was established at the center of each territory. Measurements included tree densities and diameters, density of snags >8 cm in diameter, aspect, percentage of slope, and percentages of canopy and groundcover.

We also determined distances to the closest edges from the center of each territory, from each nest, and from each quadrat, using aerial photographs, compass, and pacing. Internal edge types included open-canopy road, partially open-canopy road (including skidder trails), development (i.e. houses, buildings, etc.), river or stream, and natural canopy gap. Open-canopy roads were those that were not overtopped by trees and from which open sky was observed. Partially open-canopy roads were overtopped by trees and revealed little open sky. Natural canopy gaps were openings created by snags or windfalls. Mine–valley fill edge was considered an external edge and was measured at the territory-level only when mine was the closest edge type. When two or more edge types were approximately equidistant from the territory, the one with the least amount of canopy cover was considered the edge type (e.g. mine–valley fill over natural gap).

For each variable, the mean of quadrat measurements for each territory-mapping plot was used in statistical analyses. Non-use quadrats were those on the plot but not overlapping a territory.

Landscape Analyses

We quantified landscape characteristics by digitizing georeferenced copies of the 1996 National Aerial Photography Program (NAPP) photographs for our study areas into seven land use–landcover categories: mature deciduous forest, mature mixed coniferous–deciduous forest, grassland, barren, shrub–pole, water–wetlands, and developed. Roads, trails, and streams were overlaid on cover maps to examine territory placement in relation to canopy gaps. Fragment size was measured from aerial photographs. Final maps were corrected to reflect changes since 1996. We used the maps to calculate amounts of each cover type within 1 km of the center of each territory-mapping plot, or from the fragment center for fragments with multiple plots. We also calculated fragmentation indices (contrast-weighted edge density, core area of mature forest, area of fragment or continuous forest within 1 km of the plot center, and distance from mine edge) that may predict density of Cerulean Warblers. We calculated core area as the total area of the fragment or intact forest tract minus the area of a 100-m buffer around the inner edge of the tract. ARCVIEW (Environmental Systems Research Institute, Redlands, California) with the PATCH ANALYST extension (McGarigal and Marks 1995, Elkie et al. 1999) was used for all landscape analyses.

Statistical Analyses

Habitat models.—To develop habitat models, we used an information-theoretic approach based on the principle of parsimony, using Kullback-Leibler information and Akaike's Information Criterion (AIC) as the basis for modeling, rather than null-hypothesis testing (Burnham and Anderson 2002). With this approach, one selects a set of *a priori* candidate models on the basis of previous knowledge of the species in question, before examining the empirical data. When little is known about the system in question, a large number of models may be examined in an exploratory analysis. This method emphasizes thinking about the set of candidate models, excluding those variables that are probably not relevant to the species, and looking for potentially important variables in the literature. Models are evaluated by comparing relative AIC values among models and by examining Akaike weights (w_i) and evidence ratios (w_1/w_{i+1}) to determine the probability of each model being selected for the given data as compared with all the others (Burnham and Anderson 2002).

Microhabitat variables included in the candidate models were density of large trees (>38 cm diameter at breast height [DBH]) and snags, distance from the closest edge, and canopy cover in four height classes (Table 1). To reduce the number of candidate models, we eliminated several variables from analyses. We excluded understory stem densities, ground cover, and low canopy cover (<6 m) because all those variables were positively correlated with canopy cover >6 m and <12 m ($P < 0.001$), and negatively correlated with canopy cover >24 m ($P < 0.001$). At the landscape level, we excluded area of mature deciduous forest because it was highly correlated with core area of mature forest. Shrub–pole, grassland, wetlands–ponds, and barren were combined into one cover class (mine) because all four of those cover types

Table 1. Microhabitat and landscape variables used to model density and presence of Cerulean Warbler territories in southern West Virginia (DBH = diameter at breast height).

Variable	Code
Microhabitat	
Percentage of canopy cover:	
>6 m and <12 m	CC6-12m
>12 m and <18 m	CC12-18m
>18 m and <24 m	CC18-24m
>24 m	CC24m
Density of trees >38 cm DBH	Trees38cm
Density of snags >8 cm DBH	Snags
Distance to closest edge	DstEdge
Landscape	
Area of:	
Reclaimed mine	Mine
Mature mixed conifer–deciduous	MatMix
Development	Devel
Contrast-weighted edge density	CWED
Core area of mature forest	CoreArea
Area of fragment or continuous forest	ForArea
Distance to mine edge	DstMine

resulted from mine reclamation and because Cerulean Warblers are not likely to use any of those habitats. Landscape variables included in the candidate models were mine cover, mature mixed conifer–deciduous cover, development cover, and four fragmentation indices (Table 1).

We modeled the relationship between territory density (the dependent variable) and habitat variables (microhabitat and landscape) using Poisson regression (Stokes et al. 1995), because the frequency distribution of Cerulean Warbler territories was a Poisson distribution (Neter et al. 1988). Microhabitat data were mean values of each variable measured on all systematic vegetation quadrats within a territory-mapping plot. The experimental unit was the territory-mapping plot, except for the three fragments that contained more than one territory-mapping plot. In that case, average territory density and microhabitat data for the fragment were used in analyses. Because little is known about Cerulean Warbler habitat use in West Virginia and there is no information regarding landscape effects of mountaintop removal on this species, we proceeded with an exploratory analysis and examined a large number of candidate models ($n = 488$) using a top-down approach, by starting with the full model and deleting variables (Burnham and Anderson 2002). The full model included all 14 microhabitat and landscape variables (Table 1). We calculated AIC values with a correction factor (AIC_c), because our sample size:parameter ratio was <40 (Burnham and Anderson 2002). Models examined included all 14 univariate models, microhabitat-only models, landscape-only models, and combined models with both microhabitat and landscape variables.

To identify territory-level habitat characteristics that differentiated territories from nonuse areas, we developed logistic regression models from the seven microhabitat variables described above. Vegetation quadrats were the experimental unit. Nonuse quadrats were the 413 vegetation quadrats that fell outside of territories. Territory quadrats were the vegetation quadrats sampled at the center of each of 88 territories. Two sets of logistic regression models were developed. The first used data from vegetation quadrats in all territory-mapping plots, and the second used data only from territory-mapping plots where Cerulean Warblers were found, to exclude plots where Cerulean Warblers may not have been detected because of landscape characteristics. For each set of models, we used AIC_c values to select the 5 best models from a set of 20 candidate models that included the full model, all univariate models, and combinations of the seven microhabitat variables.

Territories in relation to treatment, slope, aspect, and edges.—We used chi-square analyses (Zar 1999) to examine territory placement in relation to treatment (fragmented and intact forest), slope-aspect position, and edge type. We then calculated Bonferroni 95% confidence intervals (Neu et al. 1974) to determine whether observed use differed from expected use based on availability within each level of a variable.

To examine treatment effects, we compared the number of territories observed in the fragmented and intact forest treatments with the number expected to occur given the amount of area (hectares) sampled. Bonferroni 95% confidence intervals around the proportion of territories observed in each treatment were then compared with the proportion expected to occur given the amount of area sampled for each treatment.

In the chi-square analysis examining territory placement in relation to slope-aspect positions, we controlled for treatment effects with a Cochran-Mantel-Haenszel statistic (SAS 1985). Number of Cerulean Warbler territories within each slope-aspect category was based on position of the territory center. We measured the area of each spot-mapping plot that was ridge, bottomland, and midslope to determine the proportion available for each. Ridge was considered the area of the plot at the peak with little or no slope. Bottomland was the area of the plot that was at the foot of the slope <25 m from a stream or creek bottom. Midslope, the remaining area between ridge and bottom, was divided into two aspect categories: northeast (0–90°) and other (91–359°). We used only two aspect categories because only 28% of midslope territories did not occur on northeast-facing slopes. The expected number of territories in each category was calculated: total number of territories × proportion of area available in each category.

In the chi-square analysis comparing use and availability of specific edge types, we again controlled for treatment effects with a Cochran-Mantel-Haenszel statistic (SAS 1985). The closest edge to each territory center and to each systematic quadrat center was identified. Use was the number of territories that fell into each closest edge type. Availability was the number of systematic quadrat centers that fell into each closest edge type. Expected total number of territories was the product of the total number of observed territories and the proportion of edge types available in each edge category. We also used Poisson regression to relate territory density with distance from mine edge.

Results

We mapped 14 territories on 175.3 ha of fragmented forest in 2001 and 10 in 2002, for an average territory density of 0.7 territories per 10 ha. In intact forest, we mapped 24 territories on 60 ha in 2001 and 40 on 80 ha in 2002, yielding a mean territory density of 4.6 territories per 10 ha.

The number of territories observed in the two treatments differed from what we expected ($\chi^2 = 85.0$, df = 1, $P < 0.01$). Fewer territories occurred in the fragments than expected given the available area of fragmented forest, and more territories occurred in intact forest than expected based on available habitat area (Table 2). Seventy-three percent of all territories occurred in intact forest, though only 28% of the total area surveyed was intact forest. Territory density was >6× higher in intact than in fragmented forest.

Territory placement varied significantly across slope-aspect categories (Cochran-Mantel-Haenszel statistic, $Q_{\mathrm{CMH}} = 24.01$, df = 3, $P < 0.001$). Territory placement was greater than expected on ridges, less than expected on midslope aspects of 91–357°, and equal to that expected on northeast aspects (Table 2). Territory density was >8× greater on ridges than on either midslope or bottom; it was almost twice as high as on northeast-facing slopes (Table 2).

Only two of seven forest fragments with no

Table 2. Cerulean Warbler use versus availability of habitat in fragmented and intact forests and different slope–aspect positions in southwestern West Virginia.

	Available area (ha)	Territories observed			Territories expected		Territories per 10 ha
		n	%	95% CI[a]	*n*	%	
Treatment							
Fragmented	350.6	24	0.27	0.18 to 0.37	63	0.72 <[b]	0.7
Intact	140	64	0.73	0.63 to 0.82	25	0.29 >	4.6
Slope position							
Ridge	105.0	44	0.50	0.37 to 0.63	19	0.21 >	4.2
Midslope, northeast (0–90°)	115.4	31	0.35	0.23 to 0.47	21	0.24 =	2.7
Mid-slope, other (91–359°)	241.8	12	0.14	0.05 to 0.22	43	0.49 <	0.9
Bottom	28.4	1	0.01	0.00 to 0.04	5	0.06 <	0.4

[a] Bonferroni 95% confidence intervals (CI) around the observed proportion of territories (Neu et al. 1974).

[b] Symbols indicate use (proportion observed) equal to the proportion expected based on availability (=); use less than availability, so avoids (<); and use greater than availability, so prefers (>).

ridge habitat (29%) had Cerulean Warbler territories; mean density was 0.17 per 10 ha. Of the eight fragments with ridges, six (75%) had territories; mean density was 0.95 per 10 ha. Ninety-two percent of all territories (22 of 24) in fragments occurred in those six fragments. Three of eight intact forest plots did not have ridges and had mean density of 0.80 per 10 ha. At least one territory occurred on every intact plot, but 92% of territories (59 of 64) were on plots with ridges (mean density = 6.0 per 10 ha). Overall, 46% of territories in fragments and 52% in intact forest were on ridges.

Territory placement in relation to closest edge type differed from the expected distribution ($Q_{\mathrm{CMH}} = 18.5$, df = 4, $P < 0.001$). Fewer territories were adjacent to streams and mine–valley fills than expected, and more territories were adjacent to partially open-canopy roads and open-canopy roads than expected given availability of those edge types within the territory-mapping plots (Table 3). Proportion of territories adjacent to natural gaps did not differ from proportion available. Most territories (63%) crossed either an open-canopy or partially open-canopy road or trail.

Habitat Models

The five best habitat models included both microhabitat and landscape variables (Table 4). All models with only microhabitat or only landscape variables had low ranks (<35) and low AIC weights (<0.01). All top five models included the same three microhabitat variables (percentage of canopy cover >6 and <12 m, percentage of canopy cover >24 m, and snag density) and the same landscape variable (distance from mine edge) as positive predictors of territory density. The best model had an AIC weight of 0.58 in relation to the other 487 models, indicating that it had a 58% probability of being chosen, given the data. The four remaining models had much lower weights (0.03–0.09). The ratio of weights

Table 3. Cerulean Warbler use versus availability of edge types in southwestern West Virginia.

Edge type	Number of systematic quadrats	Territories observed			Territories expected	
		n	%	95% CI [a]	*n*	%
Natural gap	43	10	0.12	0.03 to 0.20	8	0.09 = [b]
Stream	148	5	0.06	0.00 to 0.12	27	0.32 <
Partially open road	146	40	0.47	0.34 to 0.60	26	0.31 >
Open road	93	28	0.33	0.21 to 0.45	17	0.20 >
Mine–valley fill [c]	36	2	0.02	0.00 to 0.06	7	0.08 <

[a] Bonferroni 95% confidence intervals (CI) around the observed proportion of territories (Neu et al. 1974).

[b] Symbols indicate use (proportion observed) equal to the proportion expected based on availability (=); use less than availability, so avoids (<); and use greater than availability, so prefers (>).

[c] The mine–valley fill edge is considered a hard, external edge, whereas the other types were soft, internal edges.

Table 4. Independent variables for the five best Poisson regression models that predict Cerulean Warbler territory density in southern West Virginia, with their $\mathrm{AIC_c}$ values, $\Delta\mathrm{AIC_c}$ values, Akaike weights (w), and rank (out of 488 models). The "+" and "–" signs before each variable indicate the direction of the relationship between the variable and territory density. Variable codes used in models are defined in Table 1.

Models	$\mathrm{AIC_c}$	$\Delta\mathrm{AIC_c}$	w	w_1/w_{i+1}
+CC6-12m, +CC24m, +Snags, +DstMine	–38.46	0.00	0.58	
+CC6-12m, +CC24m, +Snags, +DstMine, –MatMix	–34.64	3.82	0.09	6.4
+CC6-12m, +CC24m, +Snags, +DstMine, +CoreArea	–34.34	4.12	0.07	8.3
+CC6-12m, +CC24m, +Snags, +DstMine, +FragArea	–32.89	5.56	0.04	14.5
+CC6-12m, +CC24m, +Snags, +DstMine, +Devel, –MatMix	–32.75	5.71	0.03	19.3

for the best model versus the four next best models ranged from 6.4 to 19.3. Distance from mine edge had no relation to territory density when all distances were plotted (Wald $\chi^2 = 1.70$, $P = 0.19$; Fig. 2A). Because only four plots were sampled at distances >400 m, we examined only plots within 400 m of the mine edge and found a strong relationship to territory density (Wald $\chi^2 = 39.02$, $P < 0.0001$; Fig. 2B). When only fragmented forest plots were included, the relationship still held (Wald $\chi^2 = 3.74$, $P < 0.05$).

To identify microhabitat characteristics that Cerulean Warblers may use for placement of territories within a plot, we developed logistic regression models comparing vegetation data from territory and nonuse quadrats. The five best models developed from all plots and only from plots with Cerulean Warbler territories all had low Akaike weights (Table 5), which indicates that those variables are poor predictors of Cerulean Warbler territory placement. Small differences between nonuse quadrats and territory quadrats for those variables (Table 6) may not be biologically significant.

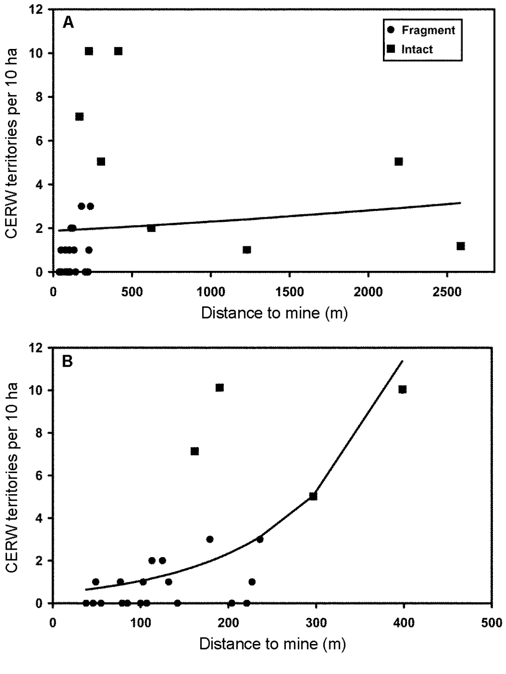

FIG. 2. Relationship between Cerulean Warbler (CERW) territory density and distance from mine edge at (A) all distances (Wald $\chi^2 = 1.70$, $P = 0.19$) and (B) distances <400 m (Wald $\chi^2 = 39.02$, $P < 0.0001$) in southern West Virginia, 2001–2002.

DISCUSSION

We found that loss and fragmentation of forests by MTMVF in southern West Virginia negatively affected populations of Cerulean Warblers through edge and area effects. Cerulean Warbler territory density was lower in forest fragments surrounded by reclaimed mine lands than in intact forests. Distance from mine edge was a significant predictor of territory density in the habitat models; density increased beyond 100 m of the mine edge, which suggests that edge effects extend ~100 m into the forest. Bosworth (2003) documented a similar response to mine edge in relative abundance of Cerulean Warblers. Territory density was low on three of the four plots >400 m from mine edge, likely because of microhabitat conditions on those three plots. Two of the plots followed stream valleys, and a third was on a south-facing slope; those sites do not appear to be optimum habitat for Cerulean Warblers, given our data and those of Bosworth (2003).

Both microhabitat and landscape components were important factors influencing territory densities. Consistent predictors of territory density

TABLE 5. The five best microhabitat logistic-regression models that predict presence of Cerulean Warbler territories in southern West Virginia, with their AIC_c values, ΔAIC_c values, and Akaike weights (*w*). The "+" and "–" signs before each variable indicate the direction of the relationship between the variable and territory presence. Variable codes used in models are defined in Table 1.

Models	AIC_c	ΔAIC_c	*w*
All plots			
+CC18-24m	467.18	0.00	0.15
+Snags	467.75	0.57	0.11
+CC18-24m, +Snags	467.81	0.63	0.11
–DstEdge	468.35	1.17	0.08
+CC24m	468.48	1.30	0.08
Only plots with Cerulean Warblers			
+CC18-24m	413.99	0.00	0.13
–DstEdge	414.00	0.01	0.13
+Snags	414.09	0.10	0.12
+CC12-18m	414.19	0.19	0.12
+Trees38cm	414.84	0.85	0.08

Table 6. Means (± SE) of microhabitat variables at territory centers in fragmented ($n = 23$) and intact forest ($n = 62$) and at nonuse quadrats (fragmented = 272, intact = 140).

	Territories		Nonuse		Combined	
Variable	Fragmented	Intact	Fragmented	Intact	Territories	Nonuse
Distance to closest edge[1] (m)	22.6 ± 6.3	33.2 ± 4.1	38.4 ± 2.5	29.5 ± 2.8	26.8 ± 3.5	38.3 ± 0.6
Percentage of canopy cover:						
>6–12 m	66.5 ± 4.4	68.6 ± 2.6	68.7 ± 1.3	64.5 ± 1.7	67.5 ± 2.2	67.3 ± 1.0
>12–18 m	69.8 ± 5.1	62.7 ± 2.7	61.5 ± 1.5	61.3 ± 1.8	64.4 ± 2.4	61.4 ± 1.1
>18–24 m	46.1 ± 6.5	45.2 ± 3.2	36.2 ± 1.8	46.2 ± 2.0	45.7 ± 2.9	39.6 ± 1.4
>24 m	8.7 ± 3.2	19.0 ± 3.0	11.3 ± 1.3	17.9 ± 1.8	16.8 ± 2.4	13.5 ± 1.1
Stem density (number per hectare):						
>38 cm	56.1 ± 9.4	46.3 ± 5.5	41.5 ± 2.1	47.2 ± 3.7	49.7 ± 4.6	43.4 ± 1.9
Snags (>8 cm)	63.0 ± 8.4	58.5 ± 7.5	48.9 ± 2.8	49.3 ± 4.7	59.7 ± 5.9	49.0 ± 2.4

[1] Any edge type, including internal soft edge and external reclaimed mine edge.

at the microhabitat level were percentage of canopy cover >6 m and <12 m, percentage of canopy cover >24 m, and snag density. Lynch (1981) found that Cerulean Warblers preferred a canopy divided into distinct vertical layers in floodplain forests of North Carolina, where tall, old-growth trees dominated the canopy. Cerulean Warblers typically nest at heights between 4.6 and 18.3 m (summarized in Hamel 2000), so it is not surprising that their territory density was higher in stands with a greater amount of canopy cover >6 m and <12 m. Preference for areas with canopy cover >24 m is in agreement with studies that found this species in areas with large, tall trees and a dense upper canopy (Lynch 1981, Robbins et al. 1992, Oliarnyk 1996). Additionally, Hamel (2000) suggested that the vertical distribution of foliage may be more important than individual values of canopy cover at different heights. Thus, it is not surprising that canopy covers at two height classes were identified as predictors of Cerulean Warbler density.

Use of areas with a high density of snags on our study plots is likely related to the apparent preference for areas with gaps in the canopy noted by other researchers (Oliarnyk 1996, Oliarnyk and Robertson 1996). Further, our data indicate that Cerulean Warblers generally were not avoiding internal edges. We often observed both males and females in or near canopy gaps, such as open and partially open trails and roads and natural tree-fall gaps. In general, Cerulean Warbler territory placement was closer to internal edge types than expected given the availability of those edge types on the plots. Two of the four nests we observed were within 10 m of a canopy gap (a natural tree-fall gap and a partially open-canopy road).

Landscape factors were also significant predictors of Cerulean Warbler territory density. Distance from mine was positively related to territory density, indicating that Cerulean Warblers are avoiding the large-scale edges produced by mines. In our habitat models, territory density was also positively associated with core area of mature forest and area of fragment, indicating a preference for large blocks of mature forest, which is similar to findings of Robbins et al. (1989, 1992). Density was negatively associated with area of mixed conifer and deciduous forest, which is not surprising, given that this species is known to be restricted to mature deciduous forests (Hamel 2000).

Results at the territory level were inconclusive. Our data indicate that there was little difference in microhabitat between territories and nonuse areas. It is possible that Cerulean Warbler habitat is not limited within the mixed mesophytic forests of southwestern West Virginia and that suitable areas are not being occupied. Males may settle where others are already present and form loose "colonies" (Hamel 2000). If that is true, then Cerulean Warblers would exhibit a clumped distribution across the landscape, and it would appear that suitable habitat is not being used. Our data suggest that Cerulean

Warblers may follow that pattern. Only 3 of 17 plots where Cerulean Warblers were present contained only a single territory.

Other studies identified large-diameter trees as being important for Cerulean Warblers (Robbins et al. 1992, Oliarnyk 1996, P. B. Hamel et al. unpubl. data). We did not find tree diameter to be an important predictor of Cerulean Warbler occurrence. We often observed clusters of territories on ridges with "small" trees (as compared with tree size in other areas of the forest). Our data suggest that tree size may be less important for Cerulean Warblers in West Virginia than in other areas. Hamel (2000) suggested that tree diameters and heights may not accurately reflect Cerulean Warbler habitat and cannot be extrapolated among areas, because those metrics are a function of topography, soils, and the site on which the forest is growing.

Both slope and aspect influenced Cerulean Warbler territory placement in our study. Territories occurred more often than expected on ridges. Territories on midslopes occurred most often on northeast aspects. Use of ridges in West Virginia has also been documented by Brooks (1908), Rosenberg et. al (2000), and Bosworth (2003). Preference for ridges could result in significant effects on Cerulean Warbler populations in the MTMVF region, because ridges are removed during mining. We suspect that loss of ridges is an important factor contributing to lower territory densities in forests fragmented by mountaintop mining. Analysis of point counts from an earlier study of MTMVF mining also indicates that Cerulean Warblers were found more often than expected at points on ridges (C. A. Weakland and P. B. Wood unpubl. data). Thus, continued removal of ridges in southern West Virginia would have negative effects on Cerulean Warbler populations.

The preference for placing territories on ridges also has implications for using Breeding Bird Survey (BBS) data to monitor populations. Most BBS routes in this part of West Virginia primarily follow valleys, where territory density is likely lowest; therefore, density or abundance estimates based on BBS data are likely underestimates. However, we have found that Cerulean Warbler abundance at off-road point counts in West Virginia generally follows a pattern similar to BBS trends, though abundance estimates cannot be compared directly (C. A. Weakland and P. B. Wood unpubl. data).

In conclusion, both landscape and microhabitat factors influenced Cerulean Warbler territory density in southern West Virginia. Cerulean Warblers preferred ridgetops within large blocks of mature forest with a high percentage of canopy cover >6 and <12 m or >24 m, and with a high density of snags. They do not appear to be avoiding internal (soft) edges, such as roads and trails, but do appear to be avoiding the external (hard) edges created by mining. From a habitat conservation perspective, it is important to recognize that mountaintop mining not only reduces the amount of forested habitat available for use by Cerulean Warblers but also lowers the suitability of the remaining forest habitat, as evidenced by lower territory density in fragmented forest and near mine edges. Loss of ridgetop habitat appears to be particularly important in reducing territory density.

Acknowledgments

Funding for this study was provided through the Species-at-Risk program of the U.S. Geological Survey (USGS). We thank staff of Arch Coal and Cannelton mining companies for logistical support and for access to their properties. Ark Land Company provided field housing. We also thank the field technicians who assisted with data collection: S. Bosworth, A. Carroll, J. Hartman, M. Jones, S. Marchetti, and J. Simmons. R. Dettmers, P. Hamel, T. Muir, K. Rosenberg, and C. Tibbott provided helpful comments on earlier drafts of this manuscript. The USGS West Virginia Cooperative Fish and Wildlife Research Unit provided field vehicles, access to computers, and logistical and administrative support. West Virginia University, Division of Forestry, also provided logistical and administrative support. Use of tradenames in this document does not imply endorsement by the Federal government.

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Associate Editor: R. L. Holberton

Attachment 24

Management guidelines for enhancing Cerulean Warbler breeding habitat in Appalachian hardwood forests

Management guidelines for enhancing Cerulean Warbler breeding habitat in Appalachian hardwood forests

Book · January 2013

18 authors, including:

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US Geological Survey at West Virginia University
144 PUBLICATIONS **1,980** CITATIONS
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James Sheehan
20 PUBLICATIONS **140** CITATIONS
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David A. Buehler
University of Tennessee
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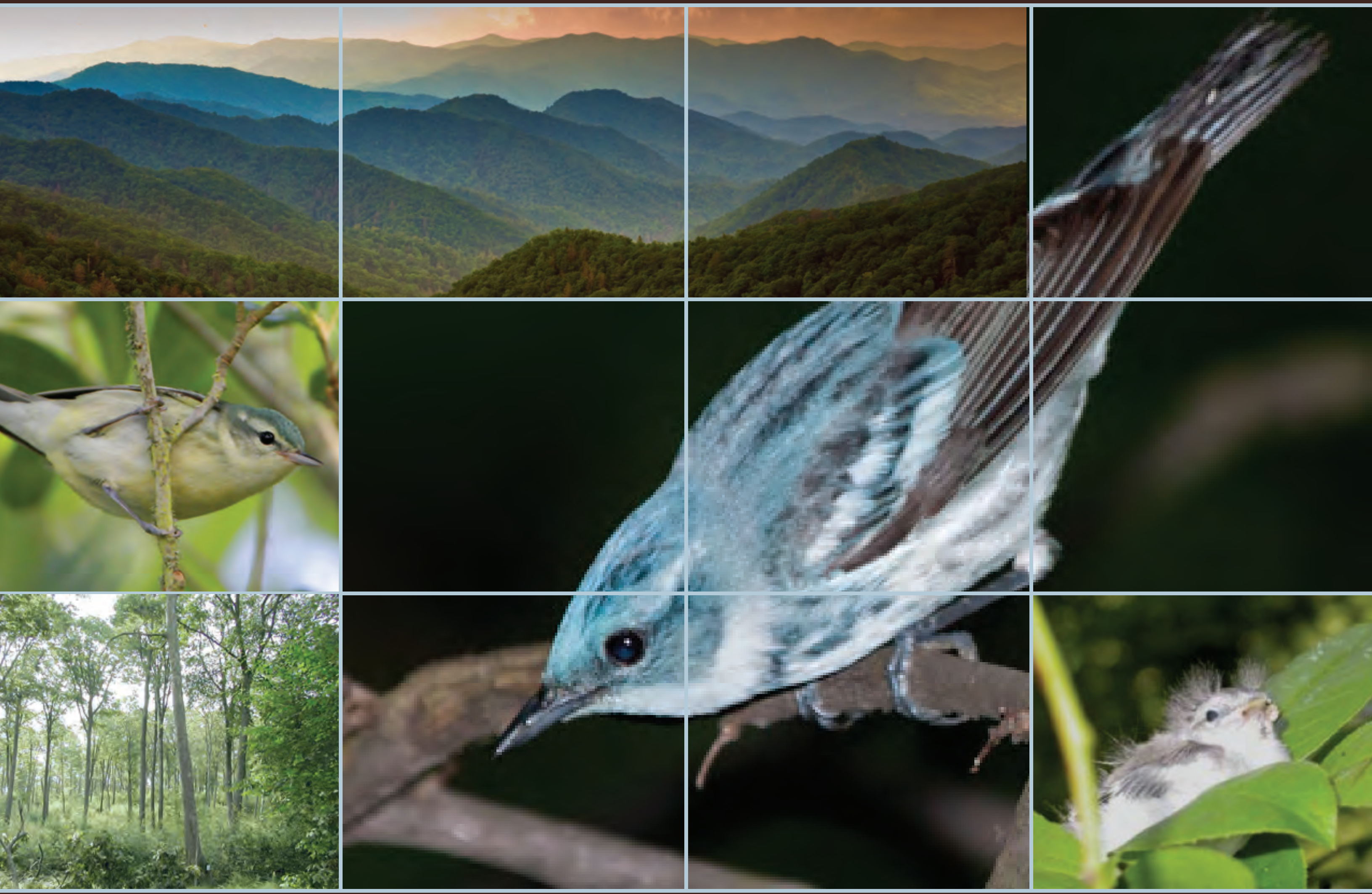
CERULEAN WARBLER

Management Guidelines for Enhancing Breeding Habitat in Appalachian Hardwood Forests



February, 2013

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Cerulean Warbler. Bill Hubick

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Publication design and layout by Debra Reynolds, USFWS

Photos: Cover: Clockwise: Appalacian landscape, Charlie Choc; Cerulean Warbler, Bill Hubick, http://www.billhubick.com; Fledgling, Marja Bakermans; Shelterwood, Scott Stoleson; Female, Bill Hubick

Appalachian breeding habitat. Than Boves

Table of Contents

Male Cerulean Warbler. Marja Bakermans

Introduction

The Cerulean Warbler (*Setophaga cerulea*) is a migratory songbird that breeds in mature deciduous forests of eastern North America. Cerulean Warblers (hereafter, ceruleans) require heavily forested landscapes for nesting and, within Appalachian forests, primarily occur on ridge tops and steep, upper slopes. They are generally associated with oak-dominated (*Quercus* spp.) stands that contain gaps in the forest canopy, that have large diameter trees (>16 inches diameter breast height (dbh)), and that have well-developed understory-and upper-canopy layers. Ceruleans primarily use the mid- and upper-canopy where they glean insects from the surface of leaves and conceal their open cup nests. Because they are severely declining across much of their range (Fig. 1), habitat management is a high priority. Management for this species can also improve conditions for a number of other wildlife species that depend on the same structure.

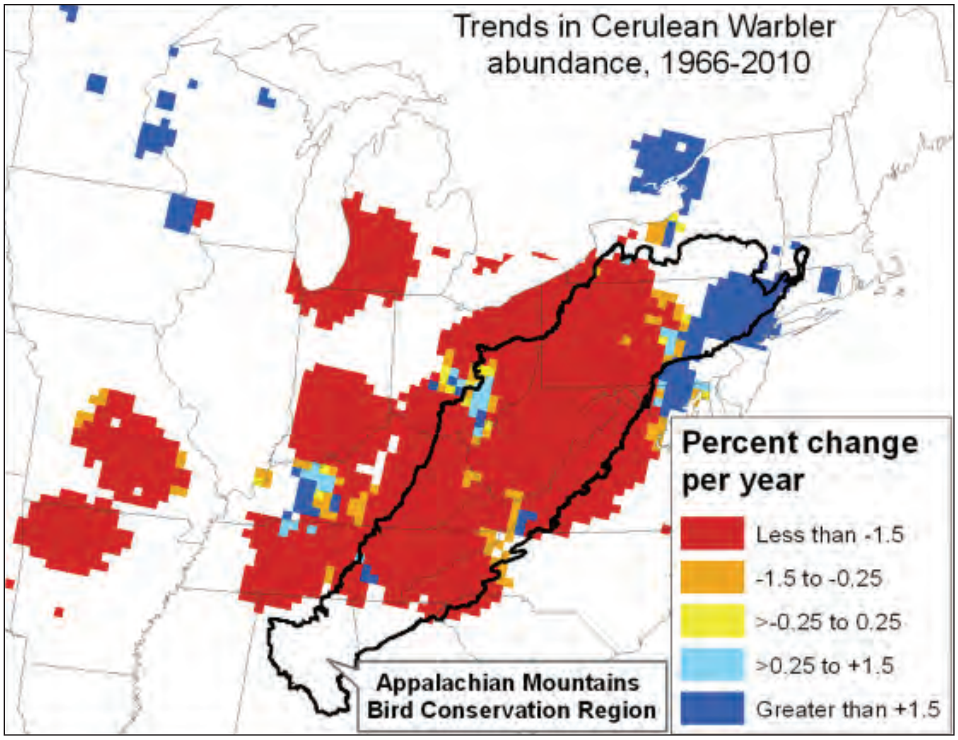


Figure 1. Cerulean Warbler distribution and trends in abundance across their breeding range from Breeding Bird Survey data (1966-2010; Sauer et al. 2011). The Appalachian Mountains Bird Conservation Region boundary is in black.

Adult Cerulean Warbler feeding chick. Wayne Miller

Goals

This document provides land managers in the Appalachian Region with guidelines for retaining and enhancing habitat for Cerulean Warblers and a diverse bird community based on the current available science. They are intended for use by federal, state and private foresters, biologists, and other land managers. These management guidelines are based to a large extent on the recently completed Cooperative Cerulean Warbler Forest Management Project (CWFMP) but also incorporate relevant findings from other research projects. All literature incorporated into this document is listed in the Reference section. The guidelines apply primarily to upland oak-dominated habitats where the majority of the research reported was completed.

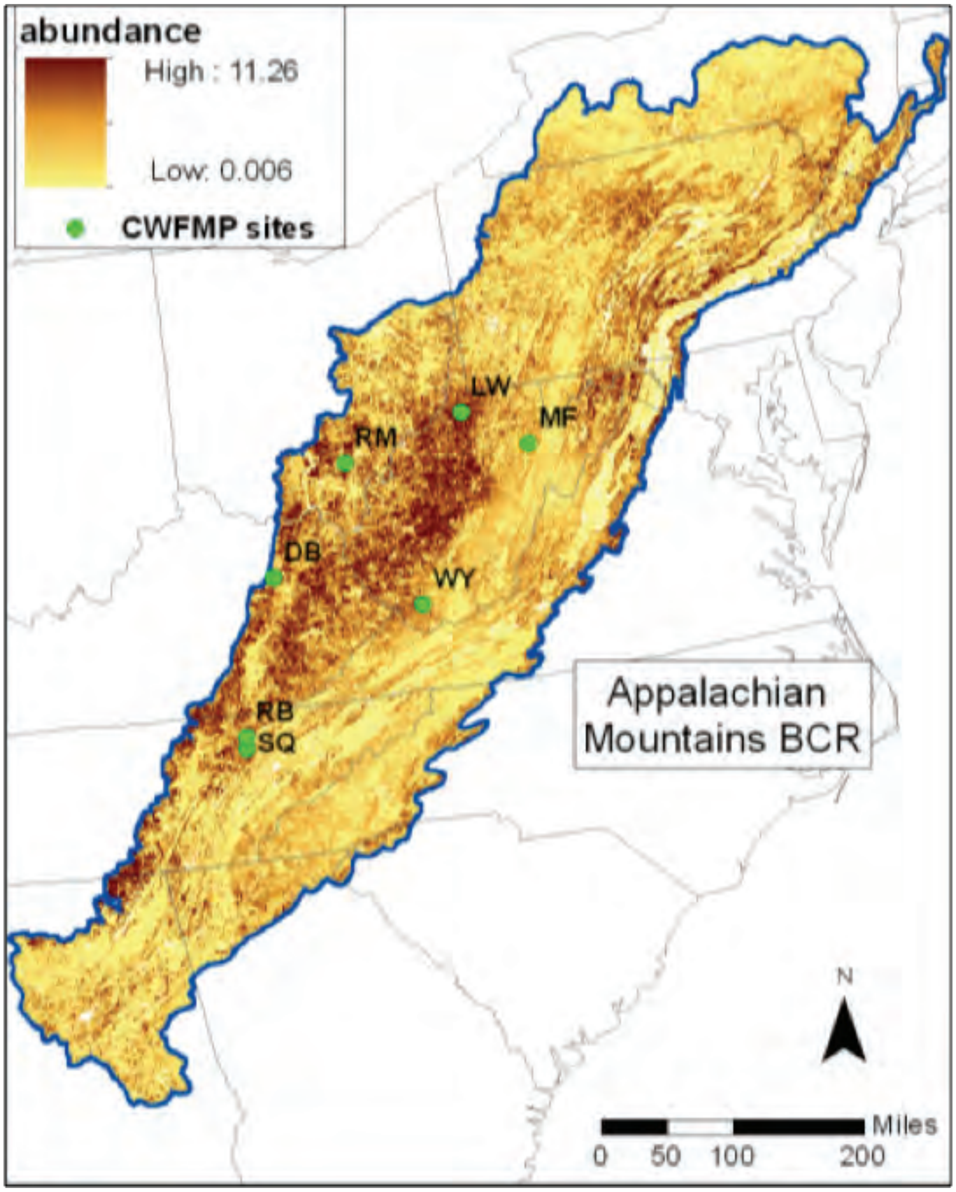


Figure 2. Cerulean Warbler abundance (number per route) estimated from Breeding Bird Survey data for the Appalachian Mountains Bird Conservation Region (BCR) (adapted from Shumar 2009). Study areas from the Cerulean Warbler Forest Management Project (CWFMP) are in the core range of the species.

Conservation

Male Cerulean Warbler. Than Boves

About 80% of the total cerulean population breeds within the Appalachian Mountains Bird Conservation Region (BCR; Fig. 1), and they are particularly abundant within the central part of the region (Fig. 2). Declines have occurred across most of their range (Fig. 1). A range-wide loss of ~70% of the population (Fig. 3) led to their designation as a species of national conservation concern by the U.S. Fish and Wildlife Service (USFWS) and as a Continental Watch List species by Partners in Flight.

Cerulean declines are primarily related to the loss and reduced suitability of habitat on breeding, migration, and wintering grounds. On breeding grounds, the second growth forests that occur throughout most forested landscapes often lack the complex forest structure favored by ceruleans. Old-growth forests naturally develop a more open and complex canopy structure, as well as multi-layered shrub and mid-story layers. Maintaining older, structurally diverse forest within cerulean breeding range may be important to sustain populations in the long-term and to support the ecosystems on which they and other organisms depend. In managed forests, however, foresters and landowners can use silviculture as a tool to develop stands with structural and compositional characteristics that are favorable for cerulean and associated species. Partial harvesting to benefit ceruleans can be consistent with forest management goals such as promoting oak regeneration and managing for a diverse wildlife community.

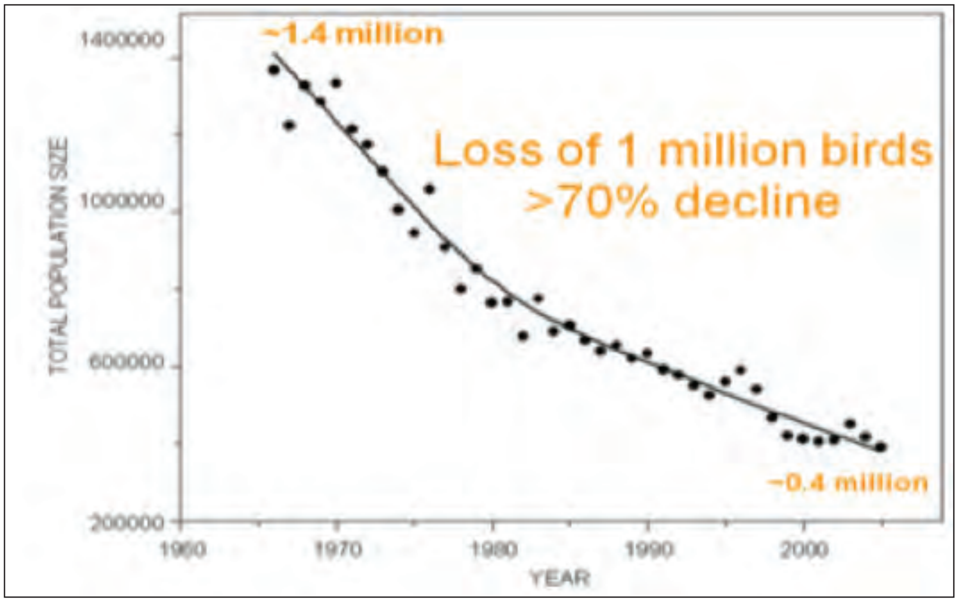
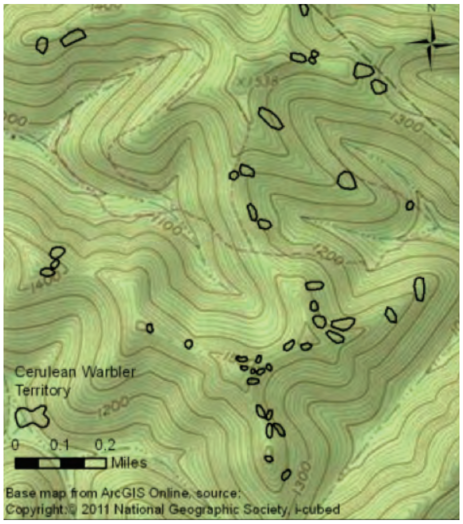


Figure 3. Cerulean Warbler population decline modeled using Breeding Bird Survey data from 1966-2006 (W. Thogmartin, unpubl. analyses).

Cerulean Warbler Habitat Association



Base map from ArcGIS Online, source:
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Figure 4. Cerulean Warbler territories on a topographic map of the Lewis Wetzel Wildlife Management Area, West Virginia, showing territories aligned along ridgelines and clustering near areas of local relief.

Cerulean breeding density is variable across the Appalachian region (Fig. 2). Their distribution is often patchy in part due to the patchy nature of canopy disturbance in mature forests and their strong association with ridge tops. In a southern West Virginia study, for example, they occurred at 40% of randomly placed sample points.

Landscape and Topography

Small forest tract size and the presence of large-scale edge (e.g., agricultural lands, mountaintop mines) can limit use of a site by ceruleans. Although the minimum forest tract size required by ceruleans to breed successfully is not known, smaller, more fragmented forest patches tend to have lower densities of territories and lower nest success. Ceruleans will use relatively small forest patches (~25 ac), but typically in landscapes that are primarily forested (e.g. >75% forest cover within ~6 miles of the project area). In landscapes with a relatively low proportion of forest cover (e.g. those that are dominated by agriculture), ceruleans are less likely to occur within small forest tracts. In the heavily deforested Mississippi Alluvial Valley, ceruleans require ~4000 acre tracts, in the highly fragmented Mid-Atlantic region ~1730 acres, and in the more forested Ohio Hills ~60 acres.

Ceruleans are often associated with canopy gaps and also use internal forest edges including narrow roads, narrow utility rights-of-way, narrow-cut strip mines, edges of small timber harvests, and trails. However, they are less abundant near abrupt or "hard" edges between forest cover and large expanses of open land (e.g., commercial, residential, and industrial development). In southern West Virginia, for example, cerulean abundance decreased near mountaintop mine edges and in northern West Virginia, they avoided edges of a large powerline right-of-way that was ~75 feet wide.

In the Appalachians, ceruleans primarily occur along ridges and steep, upper slopes and appear to cluster near areas of local relief such as knobs and bluffs (Fig. 4). The soil characteristics and topography of these features contribute to stratification of canopy trees so that ridge top forests often have a complex overstory structure containing large oaks with expansive crowns. Thus, ridge top forests often offer the structure and composition sought by breeding ceruleans. Within ridge top forests, ceruleans often favor mesic, north- and northeast-facing slopes, although other aspects are used. In some sections of the Appalachians (e.g. Delaware River valley), ceruleans are most dense at lower slope positions and along major waterways.

Appalachian landscape. Than Boves

Minimum patch size used by ceruleans depends on the amount of forest cover in the landscape.

Stand structure and Composition

Before extensive clearcutting in the late 19th and early 20th century, tree mortality from old age, windthrow, ice storm damage, and fire contributed to the development of structurally complex and relatively open stands in which oaks were dominant. In the even-aged stands that developed following those extensive harvests, natural canopy disturbances tended to be unevenly distributed and relatively small thereby creating a relatively homogenous canopy structure (e.g., a closed canopy forest with an undeveloped understory and/or midstory).

Important Components of Cerulean Habitat

Large Diameter Trees

Ceruleans place territories and nests in hardwood forests with well-spaced, large diameter trees (>16 inches dbh). Nests are typically in the largest trees available at a site.

Canopy Gaps and Structure

Ceruleans favor the complex canopy structure characteristic of uneven-aged stands and old growth forest. Canopy gaps allow mid- and upper-canopy trees the growing space to form long horizontal branches and develop dense foliage. Tree species composition is relatively diverse with shade-intolerant species abundant in the overstory.

Upland forest used by Cerulean Warbler. Marja Bakermans

Heterogenous stand structure including large trees, canopy gaps, and understory vegetation promote density and reproductive success of ceruleans.

A relatively open canopy structure provides ceruleans with dominant trees (i.e., taller than the surrounding canopy) where exposed perches aid the birds in broadcasting their song and whose expansive crowns offer ample foliage in which to forage and conceal nests. Nests are often placed along flat lateral branches that extend over a relatively open midstory and a relatively dense understory, conditions that occur adjacent to a regenerating canopy gap. Ceruleans preferentially use canopy gaps ~400-1000 ft^2 in size and that contain vegetative growth within them.

Oaks and Hickories

In the Appalachians, ceruleans are strongly associated with stands in which oaks and hickories (*Carya* spp.) predominate. They preferentially forage and nest in white (*Q. alba*) and chestnut oak (*Q. montana*), but they avoid red maple (*Acer rubrum*) and oaks from the red oak group (scarlet (*Q. coccinea*), black (*Q. velutina*), and northern (*Q. rubra*) and southern red oak (*Q. falcata*). On sites dominated by species other than oaks, ceruleans preferentially used black cherry (*Prunus serotina*) and black locust (*Robinia pseudoacacia*) in West Virginia and American elm (*Ulmus americana*) and sycamore (*Platanus occidentalis*) in Ohio for various activities.

Grapevines

Grapevines provide a favored source of nest material. Cerulean nest success was positively associated with density of grapevines (*Vitis spp.*) in Ohio perhaps because vines add complexity to the canopy and, consequently, reduce the search-efficiency of nest predators. In Maryland, fledglings often were observed perching within clumps of grapevines.

Understory Vegetation

Density and nest success of ceruleans have been positively associated with understory vegetation. In Ohio, vegetation surrounding nest locations had 24% greater understory vegetation density than random locations in the stand. A high density of understory vegetation is beneficial to ceruleans because 1) females frequently drop to the understory for intensive foraging bouts during incubation and brooding, and 2) fledgling birds often seek the dense vegetation for protection from predators.

Leave some grapevines
to provide nest material.

Female Cerulean Warbler incubating; note grapevine bark on the nest rim. This is a typical location for nests, i.e. on a lateral branch, next to a vertical twig, with an umbrella of leaves above the nest. Than Boves

Cerulean Warbler fledgling in thick understory vegetation. Marja Bakermans

Cerulean Warbler nest of grapevine and other materials. Marja Bakermans

Cooperative Cerulean Warbler Forest Management Project

The Cooperative Cerulean Warbler Forest Management Project (CWFMP), implemented under the auspices of the Cerulean Warbler Technical Group, was initiated to allow the scientific and management communities to test ideas about the habitat needs of ceruleans through experimental manipulations of timber harvest. The objective of the CWFMP was to study the response of ceruleans and the overall bird community to three silvicultural treatments and an unharvested control, collectively representing a canopy disturbance gradient. Seven study sites, each containing the four treatments, were established within mixed-mesophytic forest in Tennessee, Ohio, Kentucky, and West Virginia (Fig. 2). Sites were closed-canopy mature forest and located in heavily forested regions; forest cover within six miles of study areas averaged 83%. All stands were oak dominant.

Treatment plots were 50 acres in size and included an unharvested plot, a light harvest, a medium harvest, and a heavy harvest (Fig. 5). In harvested plots, treatments included a 25-acre harvest and a 25-acre section of undisturbed forest that bordered the harvest (hereafter buffers). Light harvests were single tree removals and residual basal area (RBA) averaged 93 ft^2/acre (range 84-106) resulting in stands that had~80% stocking. The goal of medium harvests was to thin the stand to

Pre-harvest, West Virginia LW study area, basal area = 121 ft^2/acre Patrick McElhone

Light harvest in 2007 (1 yr post-harvest), West Virginia LW study area, RBA=83.6 ft^2/acre. Patrick McElhone

Medium harvest in 2010 (4 yrs post harvest), West Virginia LW study area, RBA=45.5 ft²/acre. Jim Sheehan

Heavy harvest in 2008 (2 yrs post-harvest), Tennessee, RB study area. residual basal area (RBA)=34.5 ft²/acre. Than Boves

a residual stocking of 60-70% and favor the crown release of the best quality dominants and codominants. All other commercial stems (>6 inches dbh) were removed. The heavy harvests were applied with the objective of creating an understocked residual stand comprised of scattered dominants and co-dominants with all other commercial stems (>6 inches dbh) removed. After harvesting, the medium harvest had average RBA of 62 ft^2/acre (range 46-81) resulting in ~55% stocking. The heavy harvests had average RBA of 27 ft^2/acre (range 12-34). Basal area for unharvested plots averaged 117 ft^2/acre (range 95-138) with ~100% stocking.

The CWFMP is the largest forest management experiment ever conducted to evaluate cerulean warbler and associated songbird response to forest management. The results of the study demonstrate the initial response of ceruleans (first four years post-harvest) to forest management. Additional studies are needed to track cerulean response over the life of a managed stand to fully characterize the nature of the changes in habitat structure that occur in these stands and how ceruleans respond to these changes.

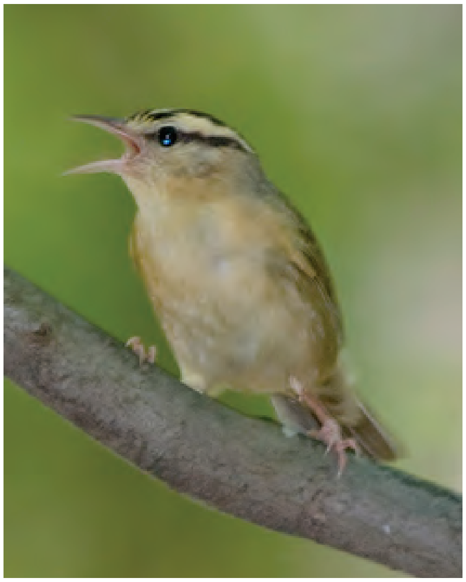
During two pre-harvest field seasons (2005-2006) and four post-harvest field seasons (2007-2010), data were collected on cerulean nest success, territory density, and habitat use. We also measured composition and relative abundance of the overall bird community to characterize response to partial harvesting and mapped territories of six other focal species in addition to Cerulean Warbler: Hooded Warbler (*Setophaga citrina*), Kentucky Warbler (*Geothlypis formosus*), Ovenbird (*Seiurus aurocapillus*), Scarlet Tanager (*Piranga olivacea*), Wood Thrush (*Hylocichla mustelina*), and Worm-eating Warbler (*Helmitheros vermivorus*).

Kentucky Warbler. Bill Hubick

Ovenbird. William Majoros

Scarlet Tanager. Bill Hubick

Wood Thrush. USFWS



Worm-eating Warbler. Bill Hubick

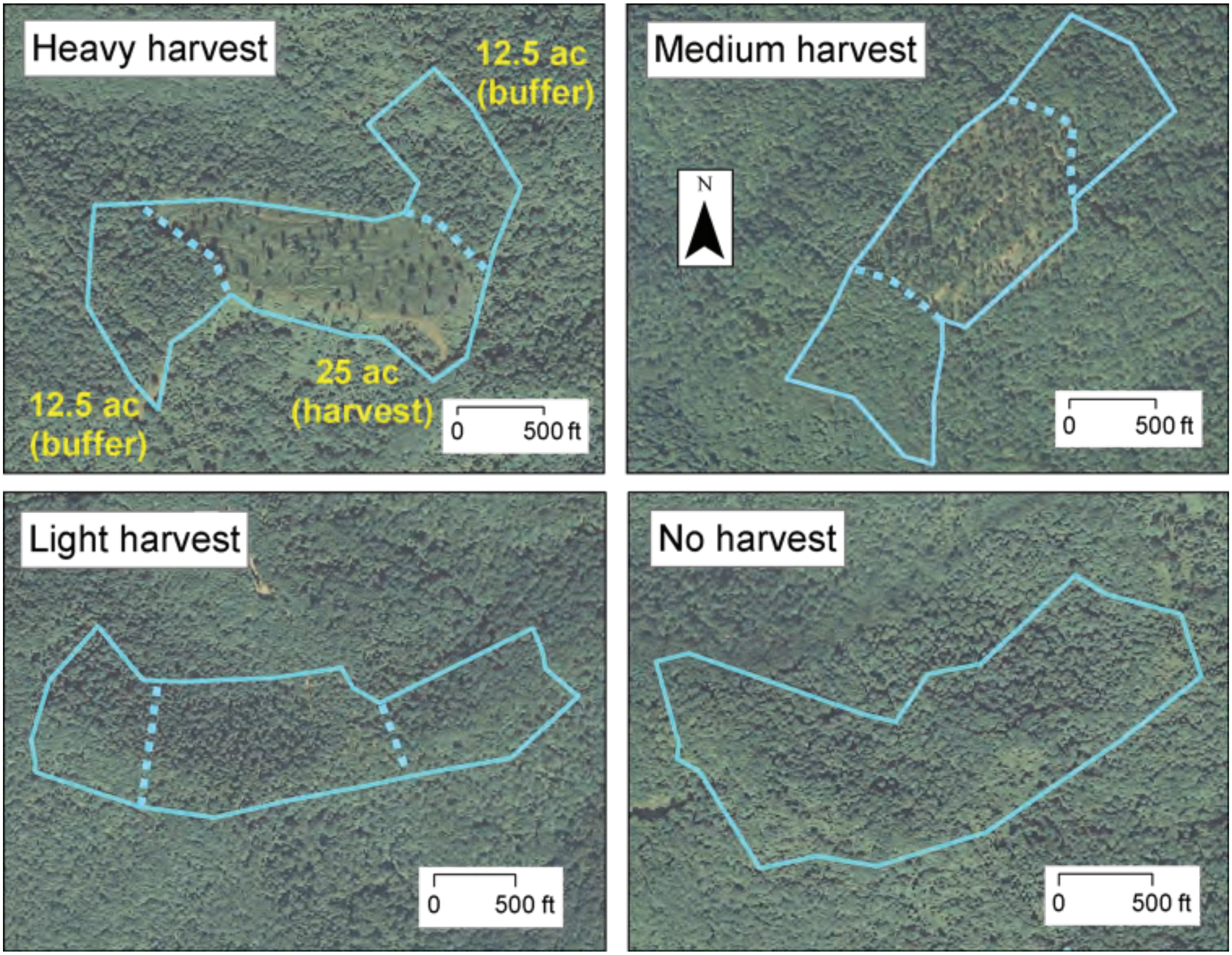


Figure 5. Plot layout in the CWFMP showing harvests and unharvested buffer areas one year after harvests were implemented on LW in WV.

Findings Relevant to Silvicultural Prescriptions

Short-term Response of Cerulean Warblers to Harvests

Territory Density

■ Across all harvests, cerulean territory density generally increased or was maintained and rarely decreased from pre-harvest densities (Fig. 6 top). The modeled response indicated that annual increases occurred (Fig. 7).

■ The largest and most consistent increases occurred when RBA was between ~40 and 90 ft^2/ac (Fig 6 top, Fig 7). An extreme increase occurred in a harvest ~45 ft^2/ac RBA where ceruleans were absent preharvest; post-harvest territories here were densely clustered.

■ Territory density increases that occurred at low levels of RBA (<40 ft^2/ac) were typically delayed 2-3 years, likely in response to the time needed for understory foliage and structural development to occur in the residual stand. Within these heavy harvests, territories were often situated along the harvest edge (Fig. 8) and nests were rarely located within the harvest.

■ Single tree selection harvests with RBA >90 ft^2/ac produced little increase in cerulean territory density (Fig 6 top).

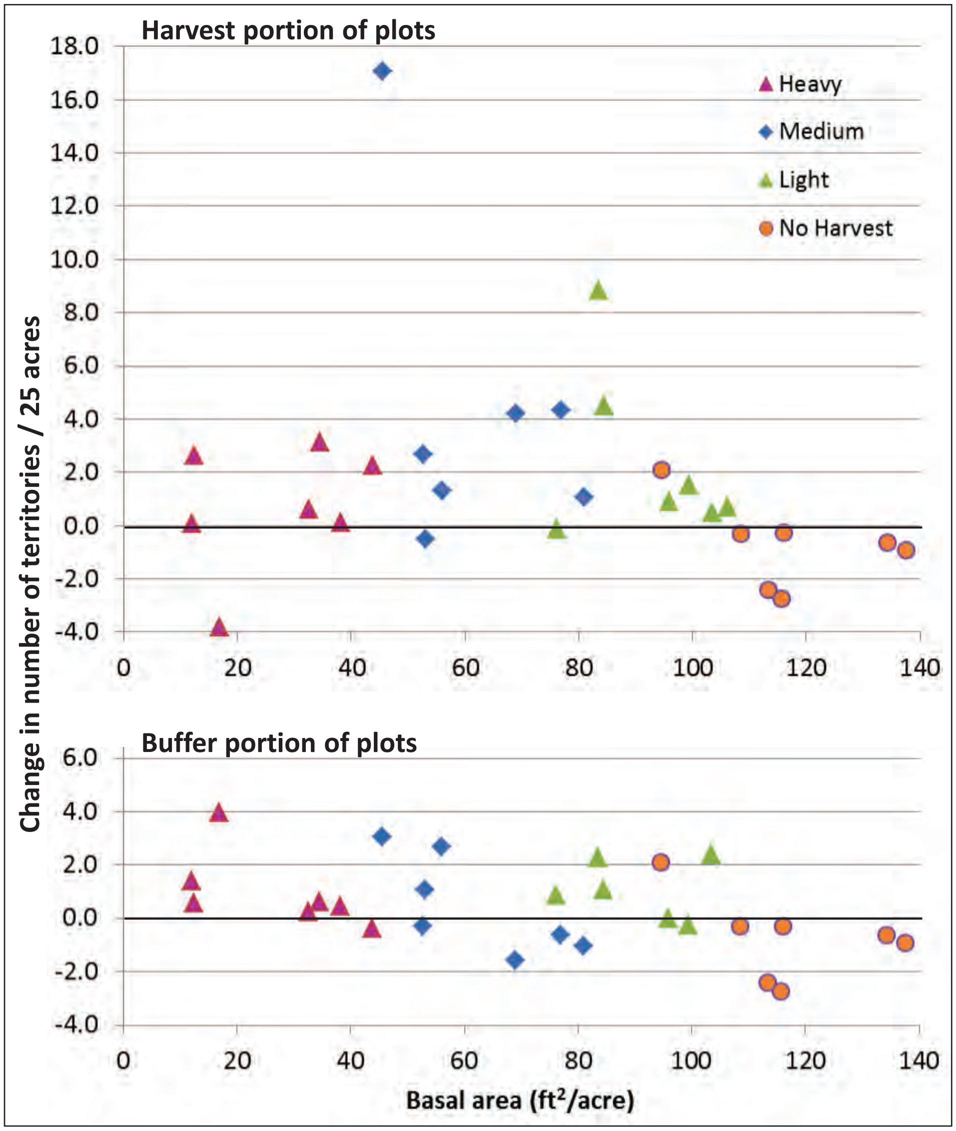


Figure 6. Mean change in number of cerulean warbler territories per 25 ac from 2006 (pre-harvest) to 2007-2010 (post-harvest) relative to post-harvest basal area and harvest intensity. Top figure is within harvests and bottom figure is within unharvested buffers. Points above the 0 line indicate plots with a mean increase in number of territories.

Ceruleans favor residual basal area of ~40 to 90 ft^2/acre of canopy trees.

■ Although the territory density response to harvests was generally positive (Fig. 6 top, Fig. 7) it was variable across study sites likely due to differences in pre-harvest cerulean densities, topography, and forest structure and composition.

■ In the majority of unharvested buffers (Fig. 6 bottom), cerulean territory density mostly increased or was maintained regardless of intensity of the adjacent harvest.

■ Some degree of thinning in the canopy of oak-dominated stands with basal area >~130 ft^2/ac would likely benefit ceruleans because territory density generally was low on these highly stocked stands (Fig 7).

Cerulean Warbler male with color bands. Matt Shumar

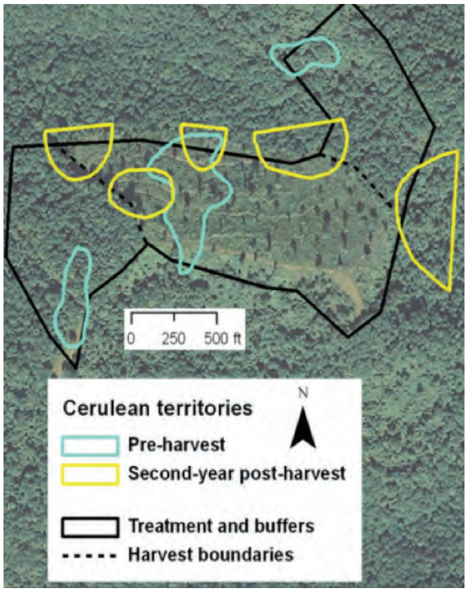


Figure 8. Cerulean Warbler territories aligned along the edge of a 20 acre heavy harvest with 12.5 ft^2/ac of residual basal area. Territories before the harvest are shown in blue and after harvest are in yellow. The birds used little of the interior of the cut.

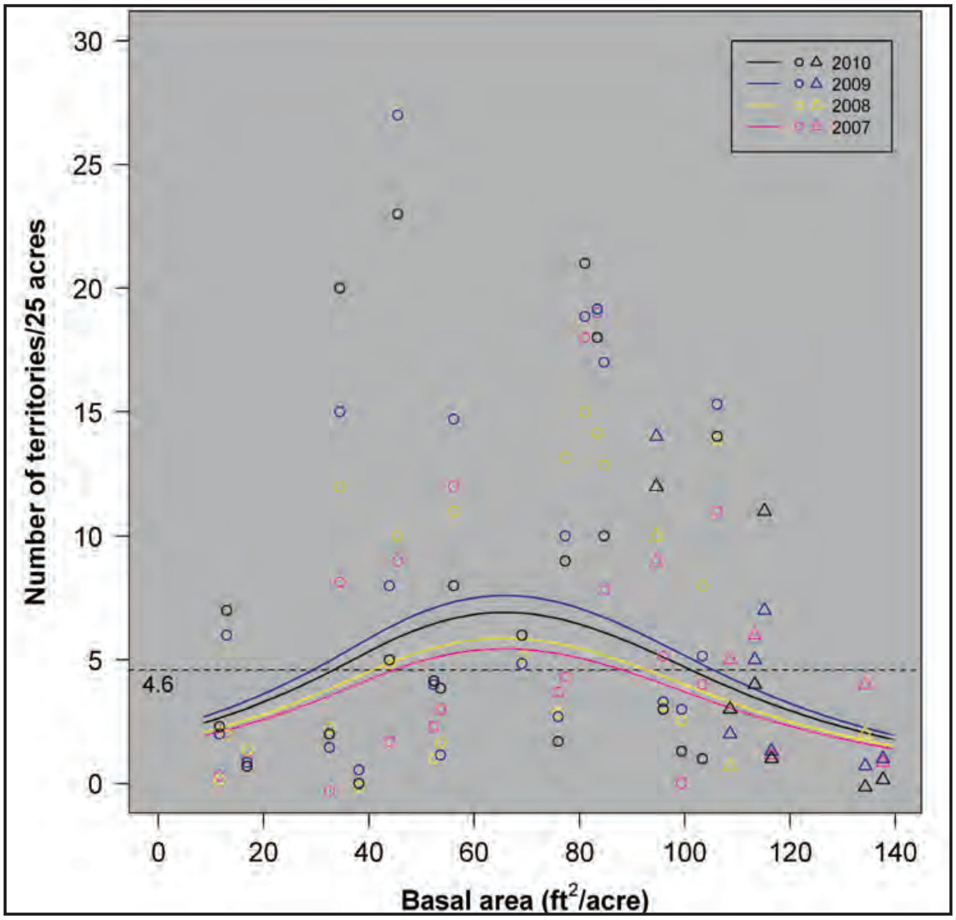


Figure 7. Annual number of post-harvest (2007-2010) cerulean warbler territories per 25 acres (circles=harvests; triangles=no-harvest control) relative to post-harvest basal area. Curved lines are the annual post-harvest predicted response for a plot with 4.6 pre-harvest territories/25 acres (the pre-harvest mean indicated by the thin dotted horizontal line).

Nest Success

- Nest success varied strongly by study site and year and was relatively low at many of the study areas. Harvest intensity had less influence on nest success than study area and year.

- Unharvested buffers adjacent to the harvests had nest success similar to that of the unharvested control stands.

- Of the three harvest treatments, medium harvests had higher nest success than light or heavy harvests (Fig. 9). However, unharvested control stands in the South region (the two Tennessee study areas) had higher nest success than any harvest.

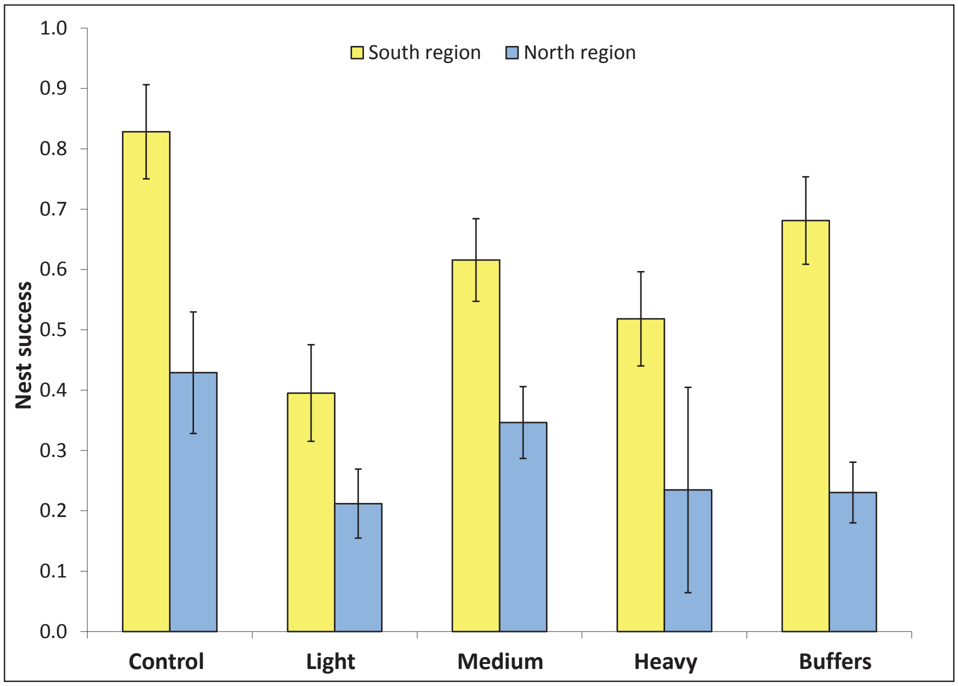


Figure 9. Cerulean Warbler nest success (with standard error bars) for the no harvest control, the three harvest treatments, and the unharvested buffers.

Male Cerulean Warbler with newly hatched chicks. Ohio DNR

Habitat Use

- For nest trees, ceruleans preferred white oak, sugar maple (*A. saccharum*), and cucumber magnolia (*Magnolia acuminata*) as nest trees and avoided red maple and oaks from the red oak group (scarlet, black, and northern and southern red oak) (Fig. 10).

- For foraging, they preferred sugar maple, chestnut oak, and hickories and again avoided oaks from the red oak group (Fig. 11).

- Ceruleans placed their nests in trees that averaged 15-19 inches dbh across the study areas. Nest trees were larger than random trees within the territory. Vegetation structure adjacent to nest trees had less mid-canopy cover and more understory cover than generally available within the surrounding territory. These conditions are characteristic of canopy gaps that have some vegetative growth within them.

White oaks, hickories, and sugar maples are favored for nesting and foraging.

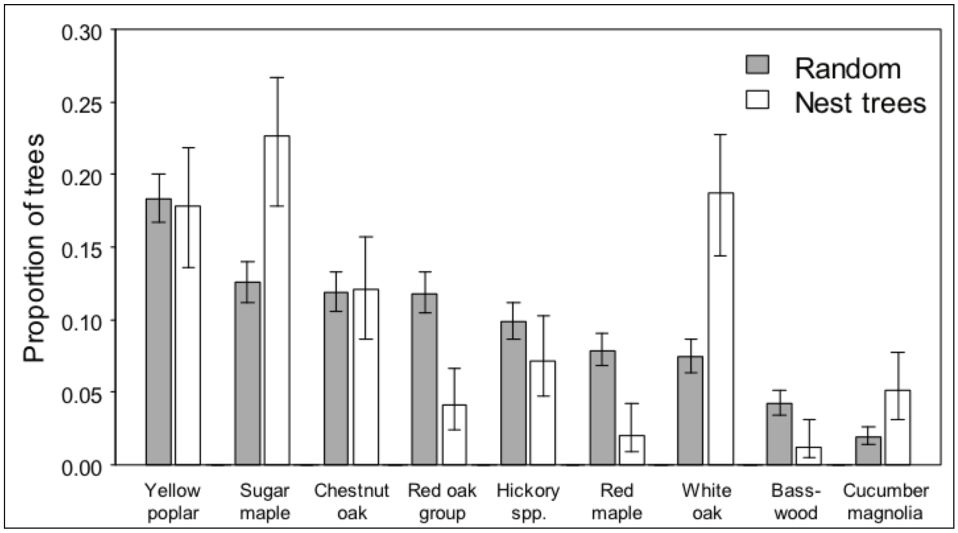


Figure 10. Nest tree selection by Cerulean Warblers at all study areas (pooled) in the Appalachian Mountains, 2008–2010. For each tree species, bars and 95% confidence intervals are the proportion of total trees within randomly sampled plots (gray) and the proportion of total nest trees (white). Red oak group includes northern red (Quercus rubra), black (Q. velutina), and scarlet (Q. coccinea) oak, and hickory species include mockernut (Carya tomentosa), bitternut (C. cordiformis), pignut (C. glabra), and shellbark (C. laciniosa) hickory. Only the most common tree species are shown.

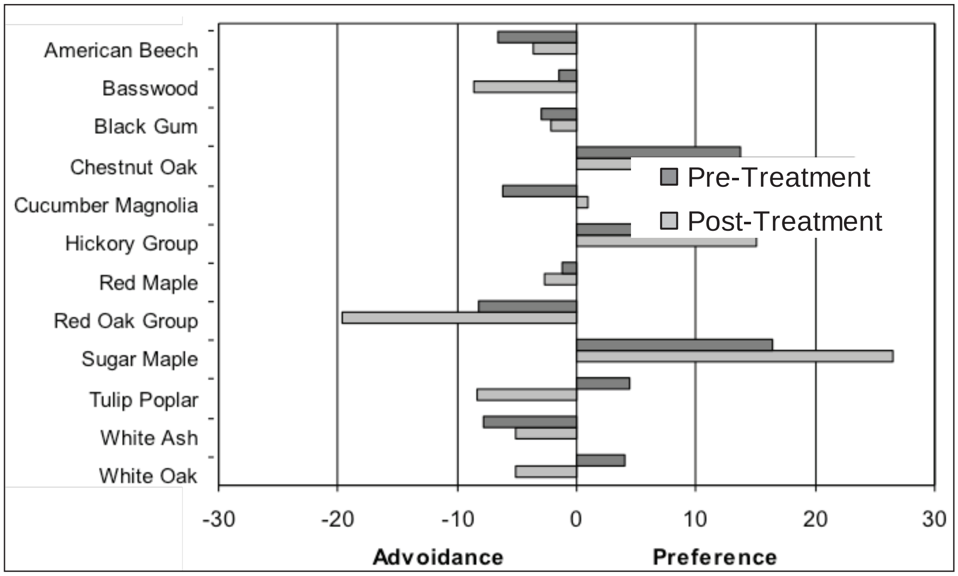


Figure 11. Pre-harvest (2006) and post-harvest (2007) indices of tree species preference and avoidance by Cerulean Warblers for the 12 most commonly available tree species.

Changes in Allied Bird Communities

Appalachian forests are considered some of the most biologically diverse temperate forests in the world. They provide breeding habitat for many avian species including those dependent on closed-canopy forest, others that require young forest habitat, and some species that require mature forest with canopy gaps. Consequently, individual species responded in various ways to different levels of RBA (Table 1).

- Ovenbird, a species that nests and forages on the ground, had its greatest abundance at high RBA (>90 ft^2/ac; Fig. 12). An immediate negative response to canopy removal persisted four years after harvests in heavy and medium harvests. Ovenbirds occurred at moderate densities in light harvests (>85 ft^2/ac).

- Species that nest in the midstory of older forests such as Wood Thrush and Acadian Flycatcher (*Empidonax virescens*), also had immediate and persistent reductions in abundance in response to canopy removal in heavy and medium harvests. This was likely in response to midstory removal and the open canopy and dense understory conditions that developed in response to these harvest levels.

- Heavy and medium harvests increased abundance and diversity of shrub-nesting species including Hooded Warbler (Fig. 12), Indigo Bunting (*Passerina cyanea*), Yellow-breasted Chat (*Icteria virens*), Kentucky Warbler, and Eastern Towhee (*Pipilo erythrophthalmus*). These species are associated with low RBA and high shrub cover. Response of some species, e.g. Hooded Warbler and Kentucky Warbler, was delayed until dense shrub cover developed.

- Certain canopy-nesting species such as Cerulean Warbler and Blue-gray Gnatcatcher (*Polioptila caerulea*) generally increased in abundance at intermediate levels of RBA across the study sites while Eastern Wood Pewee (*Contopus virens*) increased only in Ohio at intermediate RBA. Some canopy-nesters that are less sensitive to small-scale harvesting, like Scarlet Tanager, had similar abundance across the range of harvest intensities.

These short term effects are from small-scale harvesting (~25 ac) within relatively continuous mature forest. Avian species may respond differently to larger harvests, more extensive harvesting, or harvesting within landscapes with less forest cover.

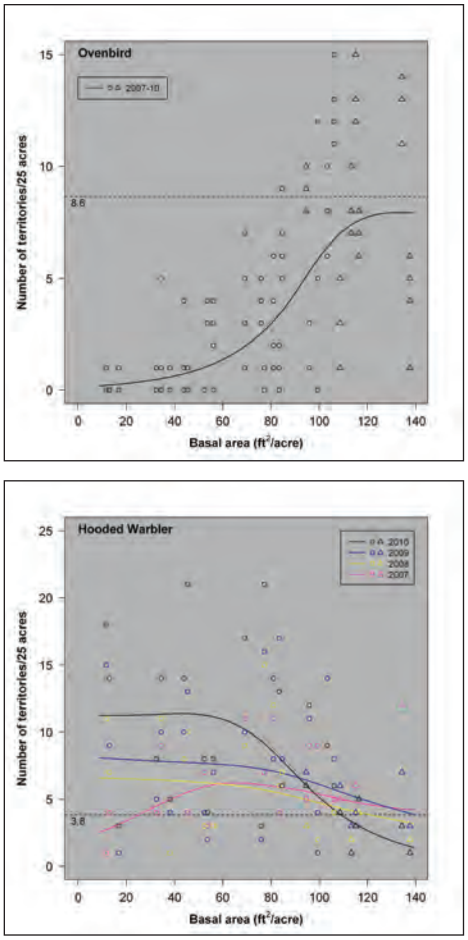


Figure 12. Number of post-harvest (2007-2010) Ovenbird and Hooded Warbler territories per 25 acres (circles=harvests; triangles=no-harvest control) relative to post-harvest basal area. Negative (Ovenbirds) and positive (Hooded Warbler) predicted responses to basal area are shown by curved lines (the pre-harvest mean indicated by the thin horizontal line). For Hooded Warbler, there was an annual increasing response during 1 to 4 years post-harvest.

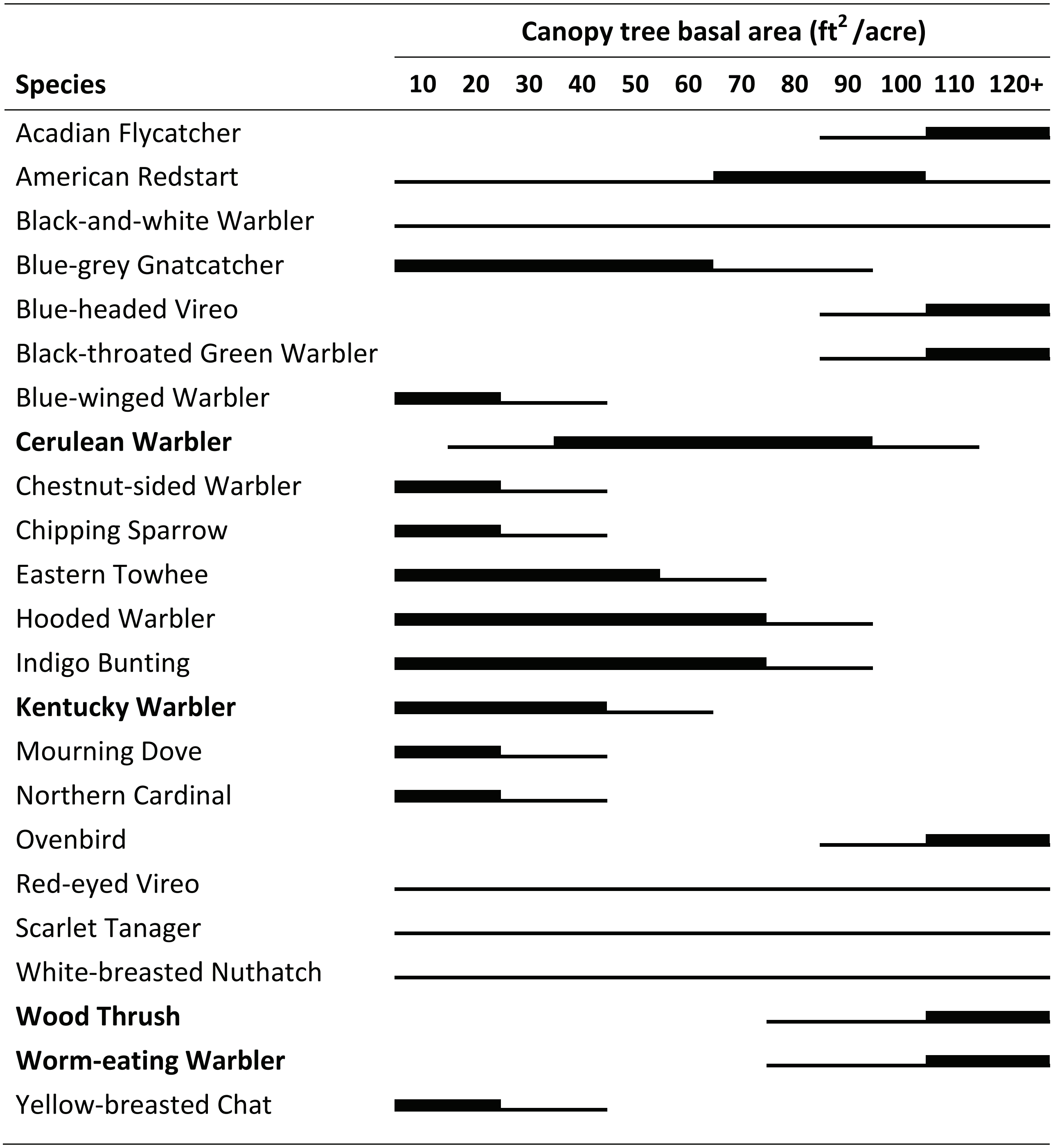


Table 1. Suitable and optimal (thickest line) basal areas for migratory songbirds that were common at CWFMP study sites. Bolded species are USFWS Birds of Management Concern. Relative abundance and/or territory density for a given species was highest under optimal basal area ranges and the species was present under suitable ranges.

Management Considerations


Female Cerulean Warbler. Ohio DNR

Cerulean Warblers occur on forested lands throughout its range. Landowners desirous of keeping their lands in forested condition can do so using the economic benefits derived from productive forest management. In mature forest stands that have high cerulean densities and high nest success, the no-harvest option is most favorable for sustaining cerulean populations. In actively managed forests, there are opportunities to use forest management practices to mimic the structure and natural disturbance regimes of old-growth forests to enhance habitat for this species. The results from the CWFMP indicate that retaining RBA levels of ~40-90 ft^2/acre after harvesting trees in 25 acre harvest units in oak-dominated stands creates a forest structure that is generally favorable for ceruleans. Small-sized harvest stands (~10-27 acres) and their edges are not avoided by ceruleans.

In addition to enhancing stand conditions for ceruleans, small-scale harvests that result in intermediate levels of RBA are consistent with promoting oak regeneration and a diverse wildlife community. These harvests create habitat for early-successional birds, many of which are experiencing long-term population declines. For example, in northeast Pennsylvania, stands of regenerating timber attract Cerulean Warblers to use both the mature forest edge and adjacent residual trees in the harvest while providing breeding habitat for Golden-winged Warblers (*Vermivora chrysoptera*). Opening the canopy also can enhance habitat for many species of forest-dwelling bats. A study of bat use of the CWFMP treatments found increased bat foraging activity within partial harvests than in unharvested plots.

Important considerations for implementing harvests for ceruleans include the following:

Landscape-scale Considerations

Forest Cover

Some studies of forest songbirds have found decreased nest success in landscapes with a low proportion of forest cover. In heavily forested regions, the abundance and productivity of ceruleans and other forest songbirds appear to be more heavily influenced by stand structure than by landscape or edge effects. Thus, habitat enhancements for ceruleans located in heavily forested regions (>70% forest cover at the six mile scale) are more likely to be effective at attracting ceruleans and landscape context may have less influence on reproductive success.

Scale of Harvesting

Even in heavily forested regions, maintaining a significant portion of the management area as mature forest cover is important for sustaining populations of forest-interior birds because many forest-interior birds are sensitive to the amount of mature forest cover at larger spatial scales. In addition, several mature forest dependent species (e.g., Wood Thrush, Worm-eating Warbler, and Acadian Flycatcher) are likely to decrease in abundance at intermediate levels of RBA. Thus, where these species are high priority, maintaining about 50% of large forest blocks in the >50 year-old age class will provide structural complexity yet retain closed-canopy forest availability.

White Oak dominated habitat. Fran Trudeau

Stand-scale Considerations

Local Cerulean Density

Where cerulean density is relatively high (>5 territories/25 acre), immediate habitat enhancements are not necessary because harvesting may reduce reproductive success which may outweigh any increases in cerulean breeding density. Ideal locations to focus management efforts are where local cerulean densities are low (<5 territories/25 acre). If no ceruleans are present near the management site (within ~5 miles), they may be less likely to colonize the managed area.

Retain large diameter white and chestnut oak trees in any management scenario.

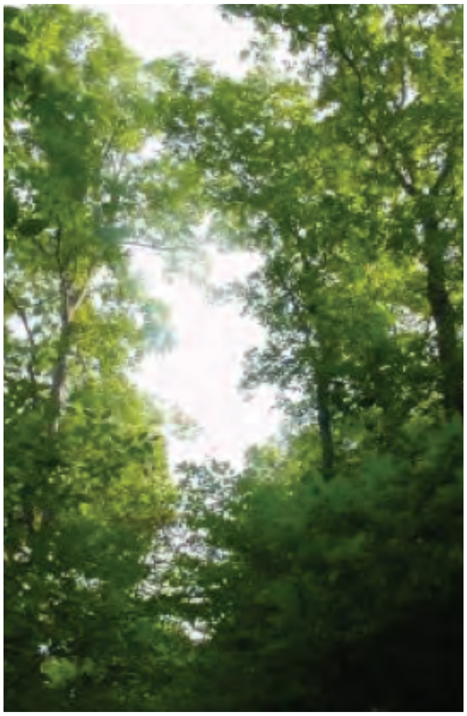
White Oak Dominance

Maintaining white and chestnut oak dominance in the residual stand is a primary consideration in implementing management strategies for ceruleans. Thus, site productivity and the presence of sufficient advance regeneration of white and chestnut oaks are important considerations in management. Where feasible, favor white oak, chestnut oak, hickories, and sugar maple in the residual stand and do not retain red maple or red oaks. Retain some of the largest diameter individuals of the preferred species as residual trees. Prescribed fire at regular intervals may be necessary to promote oak regeneration, maintain small canopy gaps, and facilitate understory vegetation diversity.

Topography

In much of the Appalachians, harvests located along ridgetops and upper slopes are likely to be more effective in attracting ceruleans. Mesic, north- and east-facing slopes are often favored by ceruleans although other aspects are used.

Size of Canopy Gaps
Ceruleans preferentially use canopy gaps that are ~400-1000 ft^2 in size, particularly those with advanced vegetative growth within them. Thus, group-selection harvests that allow already established regeneration to grow into a stratified canopy may benefit this species.



Canopy gap in West Virginia.
Scott Bosworth

Temporal and Silvicultural Considerations

A number of different silvicultural practices could achieve residual basal areas in the harvested stand that are suitable for cerulean warblers (~40-90 ft^2/acre). Some additional considerations for various silvicultural treatments are below.

■ *Single-tree selection harvests* (our light harvest treatment) were less effective in increasing cerulean numbers and rapid canopy closure may limit the duration of suitable habitat. Single-tree selection with RBA above ~90 ft^2/acre also led to lesser nest success than harvests with lesser RBA. However, if single-tree harvest is favored by a landowner for providing income, cerulean densities would still be maintained particularly if non-preferred trees are removed and preferred oaks are retained.

■ *Group selection as part of an uneven-aged system* can improve cerulean habitat and would likely be effective longer than single-tree selection. The small group openings provide for diverse canopy structure and understory development. This approach has been shown to advance stands toward late successional structure beneficial to many avian species.

■ *Shelterwood harvests* are often compatible with promoting oak regeneration and, in the CWFMP, generally resulted in increased cerulean density and intermediate levels of nest success. However, complete overstory removal during the second stage of a shelterwood harvest will substantially reduce numbers of mature forest species including Cerulean Warbler, Wood Thrush, Acadian Flycatcher, and Worm-eating Warbler. If managing for forest birds, retain the residual canopy as long as possible and until adjacent habitat has been enhanced with shelterwood or other types of harvests and colonized by ceruleans.

■ *Thinnings* as part of intermediate harvest treatments would open the canopy and provide the structure favored by ceruleans. These could take the form of a crown thinning or shelterwood seed cut.

Shelterwood harvest. Scott Stoleson

- *Modified even-age regeneration* can be used to create future opportunities for cerulean habitat improvement. Leaving large-diameter residual stems in a harvest unit can lead to development of two-aged stands. Such stands achieve more complex canopy structure earlier in their development than similar single-aged stands and the residual stems allow for some use of the stand by forest birds. Ceruleans had increased density in RBA of >~40 ft^2/acre.

- *Crop-tree release* is a practice that is used to accelerate development of crop-trees on higher quality sites. The practice is typically applied in 15 to 20 year-old stands. It can allow for earlier canopy differentiation by accelerating growth of dominant stems. Impact on habitat suitability for ceruleans will not be immediate, but benefits should be seen as the stand develops and where earlier entry into the stand for commercial harvest is made possible.

Complex canopy structure in a deferment cut creates future opportunites for Cerulean Warbler habitat improvements. Doug Becker

Summary

Forest management that incorporates these guidelines and that is applied to oak-dominated stands in the Appalachian region can enhance habitat for Cerulean Warblers and other avian species, as well as other wildlife. Managers can choose a range of residual basal area targets depending on their priority avian species of interest.

For ceruleans, the RBA target range of ~40-90 ft^2/acre results in the most increases for the longest time period. A variety of silvicultural approaches can achieve this range. Where cerulean densities are high (>5 territories/20 acres), habitat management is not likely to be needed.

Landscape considerations are also important. These recommendations may be most beneficial in areas with high forest cover. They have not been tested in landscapes where forest cover is low.

Sitting pretty. Bill Hubick

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Appalachian landscape. Charlie Choc