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GEOGRAPHIC PATTERN ANALYSIS OF PESTICIDE EXPOSURE IN SALAMANDER POPULATIONS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

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***Abstract.*—Pesticides are one of several environmental stressors likely to be involved in global amphibian declines. Numerous studies have demonstrated that very low levels of pesticides may have significant negative biological effects on amphibians. Evidence of aerial transport of pesticides over considerable distances has raised concern for pristine wilderness and preserved areas, including national parks. We investigated geographic patterns of pesticide residues in Black-belly Salamanders *Desmognathus quadramaculatus* (Plethodontidae) in the Great Smoky Mountains National Park (GSMNP). In 2001, we conducted a pilot study, collecting tissue samples from two sites (north and south of the eastern continental divide) near the western boundary of the GSMNP. At the northern site, the samples revealed detectable levels of DDT metabolites and the organophosphate chlorpyrifos. In 2003, we conducted a large scale geographic analysis of pesticide residues in Black-belly Salamanders by collecting tissue samples from the same two sites and an additional six sites, forming two east-west transects on either side of the continental divide. The results revealed measurable levels of numerous pesticide residues, including DDT and its metabolites, as well as chlordane, heptachlor, endosulfan, and atrazine. The geographic pattern was quite heterogeneous; however, samples from the two most western sites did exhibit the greatest variety of pesticide residues. Our study indicates that amphibians within the GSMNP are being exposed to both historical and current use pesticides. These environmental stressors may therefore pose significant risks to some amphibian species within the park.**

***Key Words*.—**amphibians; Black-belly Salamander, DDT, *Desmognathus quadramaculatus*, Great Smoky Mountains National Park; pesticides

INTRODUCTION

There is accumulating evidence that very low concentrations of herbicides and pesticides negatively impact amphibians (Kiesecker 2002). Several studies demonstrate feminization of male frogs exposed to trace amounts of the herbicide atrazine (Hayes et al. 2002); whereas, low level exposures to the organophosphate insecticide chlorpyrifos can induce developmental abnormalities and depress nervous system activity in amphibians (Mazanti 1999; Sparling et al. 2001). Low levels of endosulfan and atrazine also affect growth and behavior of larval salamanders (Rohr et al. 2003). Another concern is evidence for summertime aerial transport of volatilized chlorpyrifos and similar pesticides over considerable distances into pristine wilderness areas, including national parks (Lenoir et al. 1999). Fifty percent of Pacific Chorus frogs (*Hyla regilla)* from the Sierra Nevada (including Yosemite and Sequoia National Parks) contained residues of up to 190 ppb of chlorpyrifos and/or diazinon (Sparling et al. 2001). The southern Appalachians, including the Great Smoky Mountains National Park (GSMNP) are a center of salamander evolution and diversity (Dodd 2004), with the GSMNP containing 31 salamander and 13 frog species. National parks can provide critical habitat and refuge from anthropogenic stressors; however, the GSMNP is notorious for poor air quality (Johnson and Taylor 1989; Mueller 1994; National Park Service. 2002. Air Quality in the National Parks, 2nd ed. Available from http://www2.nature.nps.gov/air/pubs/aqnps.cfm [Accessed on 14 June 2007]) and significant acid deposition (Day et al. 1997), from coal power stations, automobiles, and other industrial sources (Jim Renfro, Air Quality Specialist, National Park Service, pers. comm.). We tested whether amphibian populations in the GSMNP are susceptible to pesticide deposition from upwind farmland, and described any present geographic deposition patterns. The wind can carry volatilized pesticides from spring crop plantings for long distances (Lenoir et al. 1999) allowing them to travel to distant ecosystems by wet (McConnell et al. 1998) and dry deposition (Majewski et al. 2006. Contribution of Atmospheric Deposition to Pesticide Loads in Surface Water Runoff. USGS Open-File Report 2005-1307. Available from http://pubs.usgs.gov/of/2005/1307/ [Accessed 7 August 2007]). Spring and summer prevailing winds are predominantly southwesterly in the southern Appalachians (National Climatic Data Center. 2007. Climate Maps of the United States. Available from

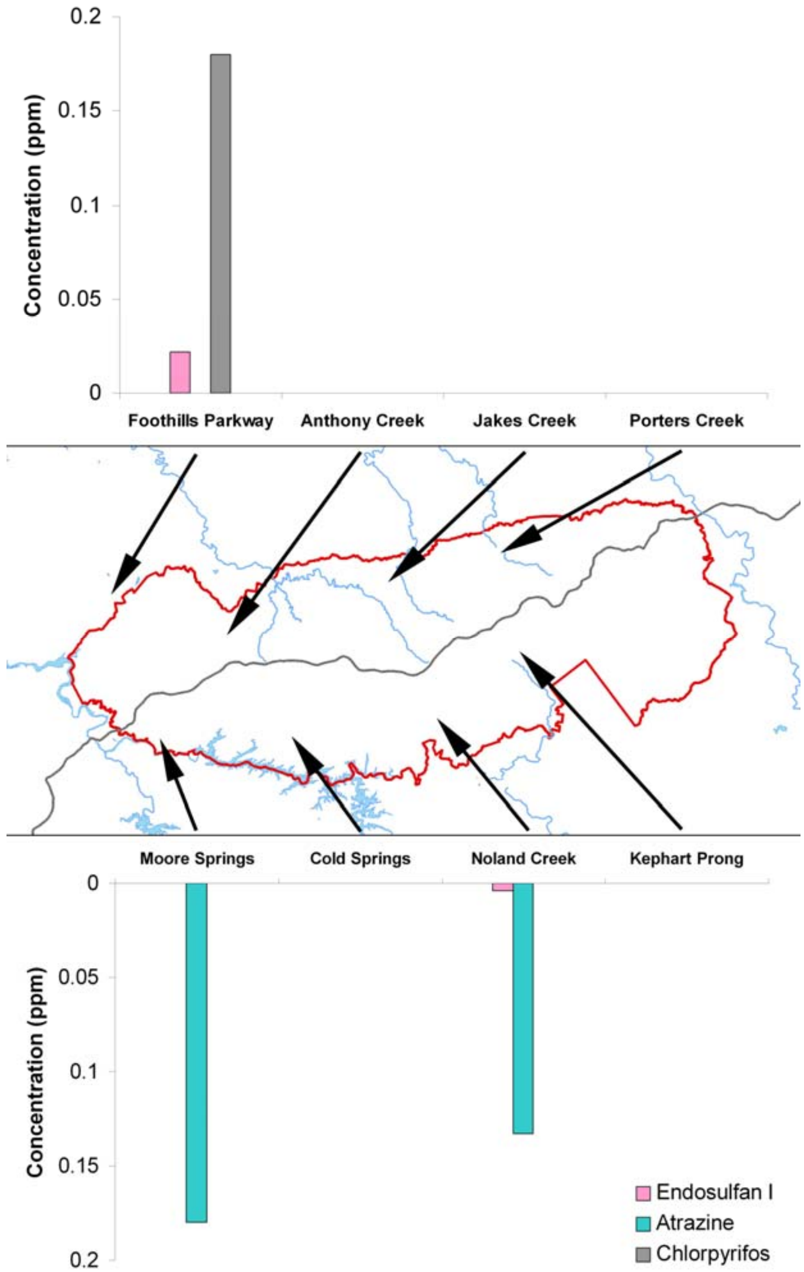

FIGURE 1.—Maximum concentrations of current-use pesticide residues identified in lipid tissue from the tail of *D. quadramaculatus* salamanders from transect sites in Tennessee and North Carolina, USA during 2001 and 2003. Arrows point to the location of the survey sites. Major rivers are indicated in blue, the national park boundary in red, and the Tennessee/North Carolina state border in grey.

http://cdo.ncdc.noaa.gov/cgi-bin/climaps/climaps.pl [Accessed 14 June 2007]); therefore, we expected a decreasing gradient of pesticide exposure from west to east across the GSMNP

MATERIALS AND METHODS

We used Black-belly Salamanders *Desmognathus quadramaculatus* (Plethodontidae) as our model because they are a large streamside salamander that is abundant and widely distributed throughout the park (Dodd 2004). Because they often eat other salamanders (Dodd 2004), they may be relatively susceptible to biomagnification of some pesticides.

Study Sites.—In 2001, we conducted a pilot study, collecting salamander tail tissue from two sites – Foothills Parkway (UTM 17S 0230438E 3944939N,

TABLE 2.— Mean concentrations of agrichemical residues identified in tail lipid tissue of *D. quadramaculatus* salamanders from transect sites in Tennessee (2003). Range of concentrations is given in parentheses.

	Lipid pesticide concentration range (ppm)			
Agrichemical	Foothills Parkway	Anthony Creek	Jakes Creek	Porters Creek
α-chlordane	0.002 (0-0.005)	0	0	0
γ-chlordane	0.023 (0-0.06)	0	0	0
DDD p,p'	0.005 (0.005-0.005)	0	0	0
DDE p,p'	0.009 (0-0.017)	0	0	0
DDT p,p'	0.019 (0.007-0.033)	0.044 (0.028-0.062)	0	0.01 (0-0.031)
Dieldrin	0.0137 (0- 0.006)	0	0	0
Endosulfan I	0.014 (0.005-0.022)	0	0	0
Heptachlor	0	0	0.001 (0-0.004)	0

elevation 600m) and Moore Springs (17S 0239753E 3930265N, elevation 560m). The sites are located north and south of the eastern continental divide forming the Tennessee/North Carolina state border, near the western boundary of the GSMNP (Fig. 1). In 2003, we conducted a larger scale geographic analysis by collecting salamanders from the same two sites and an additional six sites, forming two east-west transects of about 55 km in length on either side of the continental divide (Fig. 1). The sites in Tennessee were Anthony Creek (17S 0249224E 3943509N, elevation 600m), Jakes Creek (17S 0265738E 3946308N, elevation 900m) and Porters Creek (17S 0284107E 3953064N, elevation 900m). The sites in North Carolina were Cold Spring Branch (17S 0261104E 3930960N, elevation 1160m), Noland Creek (17S 0273061E 3931145N, elevation 800m) and Kephart Prong (17S 0285952E 3940994N, elevation 870m).

Tissue analysis.—At each site, we collected 12 specimens of adult *D. quadramaculatus* by turning rocks along the stream edge. Tissue samples were collected either by clipping tails from salamanders, or by collecting whole specimens, which were euthanized with dry ice. We used tail tissue in order to ensure consistency with the pilot study. At that point, the National Park Service preferred to avoid sacrificing specimens until there was evidence for significant pesticide residues. The samples were frozen at -20°C and delivered to the Virginia Tech Pesticide Residues Lab. For each site, the 12 salamanders were pooled into three groups of four animals. The tail tissues were blended within each group, producing three samples for analysis per site.

Samples were extracted using matrix solid phase dispersion (Long et al. 1991; Barker 2000; Linzey et al. 2003). We homogenized and extracted the samples in a single step with surface-modified bonded silica sorbent (C-18) using a mortar and pestle, and then transferred to a syringe barrel column containing pre-packed Florisil®, and eluted with methylene chloride. Pesticide analysis employed an Agilent model 6890 gas chromatograph to identify the following pesticides: Aldrin, alpha-BHC, alpha-chlordane, atrazine, carbofuran, chlordane, chlorpyrifos, o,p-DDTs (3), p,p-DDTs (3), diazinon, dicofol (Kelthane), dieldrin, endosulfan, endrin, gamma-BHC (Lindane), gamma-chlordane, heptachlor, heptachlor epoxide, hexachlorobenzene, methoxychlor, metolachlor, oxychlordane. Analytical standards came from various sources including Restek Corporation, Chem Service Inc., and the EPA repository. We prepared calibration standards in hexane using serial dilution. We made extracts from fortified (spiked) salamander and locally purchased frog tissue and based reportable levels on the lowest spike concentration with a reasonable recovery. Minimum threshold for detection was 0.004 ppm.

TABLE 1.— Mean concentrations of agrichemical residues identified in tail lipid tissue of *D. quadramaculatus* salamanders from pilot study sites in Tennessee and North Carolina (2001). Range of concentrations is given in parentheses.

	Mean Lipid pesticide concentration (ppm)	
Agrichemical	Foothills Parkway	Moore Springs
Chlorpyrifos	0.137 (0.099-0.160)	0
DDE p,p'	0.013 (0-0.02)	0

RESULTS

We detected various agrichemicals in the park. We detected the organophosphate chlorpyrifos only during the pilot study at Foothills Parkway (Table 1). DDT and its metabolites occurred in tissue samples at Foothills Parkway, Anthony Creek, Porters Creek, Moore Springs and Cold Spring Branch (Tables 2 and 3). Other organochlorines included alpha- and gamma-chlordane (Foothills Parkway and Moore Springs), heptachlor (Jakes Creek, Moore Springs and Noland Creek) and dieldrin (Foothills Parkway) (Fig. 1 and 2). The cyclodiene pesticide endosulfan occurred at Foothills Parkway and Noland Creek. We also found residues of the triazine herbicide atrazine at Moore Springs and

TABLE 3.—Mean concentrations of pesticide residues identified in lipid tissue from tails of *D. quadramaculatus* salamanders from transect sites in N. Carolina (2003). Range of concentrations is given in parentheses.

	Lipid pesticide concentration range (ppm)			
Pesticide	Moore Springs	Cold Spring Branch	Noland Creek	Kephart Prong
γ -Chlordane	0.001 (0-0.004)	0	0	0
DDD p,p'	0.013 (0.008-0.018)	0	0	0
DDE p,p'	0.001 (0-0.003)	0.002 (0-0.005)	0	0
Endosulfan I	0	0	0.001 (0-0.004)	0
Heptachlor	0.001 (0-0.004)	0	0.001 (0-0.004)	0
Atrazine	0.06 (0-0.18)	0	0.044 (0-0.133)	0

Noland Creek.

DISCUSSION

The salamander tail lipid samples revealed the presence of numerous types of pesticides. Most of these pesticides (DDT, chlordane, heptachlor) have been banned in the United States since the 1970-80s (Sparling 2000). These residues are therefore indicative of very persistent and pervasive contamination, typical of most ecosystems worldwide (Sparling 2000). The levels we observed are probably insufficient to produce significant deleterious biological effects (Donald Sparling, pers. comm.). Nevertheless, it is interesting that many of these pesticides did not appear in the 2001 tissue analyses. Indeed, we identified no pesticide residues of any kind at the Moore Springs site in 2001. This suggests significant temporal variation in exposure and/or uptake of persistent pesticides by salamanders.

We found measurable residues of currently used pesticides. Chlorpyrifos occurred at the Foothills Parkway (Tennessee) in 2001, endosulfan was at the Foothills Parkway (Tennessee) and Noland Creek (N. Carolina), and atrazine was at Moore Springs and Noland Creek (both in N. Carolina). Chlorpyrifos is an organophosphate with moderate but variable toxicity to amphibians. LC50 values range from 1-3000 µg/L for *Bufo americanus* and *Rana pipiens* tadpoles respectively (Cowman and Mazanti 2000). It is one of the most widely used crop insecticides in the US (Mazanti 1999; Cowman and Mazanti 2000). Sparling et al. (2001) found similar levels of chlorpyrifos in Pacific Chorus Frogs collected at sites in the Sierra Nevada; and chorus frogs had depressed cholinesterase activity, demonstrating a significant biological effect of the pesticide residues at these low concentrations. Endosulfan is a cyclodiene and is highly toxic to amphibians. LC50 values range from 1.8 µg/L for *Rana tigrina* (Gopal et al. 1981) to 123 µg/L for *Bufo melanostictus* (Vardia et al. 1984). It is used primarily on a variety of food crops, and is characterized by low solubility in water and moderate persistence in soil (EPA. 2002. Reregistration Eligibility Decision for Endosulfan. Available from http://www.epa.gov/oppsrrd1/ REDs/endosulfan_red.pdf [Accessed 14 June 2007]). There is considerable evidence for lethal and sublethal effects in amphibians at levels as low as 0.03-1.3 µg/L, including behavioral and developmental abnormalities (Berrill et al. 1998; Broomhall and Shine 2003; Park et al. 2001; Rohr et al. 2003). Atrazine is the most widely used agricultural herbicide used in the US (Hayes et al. 2003) and is moderately persistent in soils (half life is 60 - 100 days (Wauchope et al. 1992), with moderate solubility in water (Wauchope et al 1992). Atrazine has relatively low toxicity in amphibians; LC50 values range from 0.41mg/L for *Rana catesbeiana* to 127 mg/L for *Bufo americanus* (Birge et al. 2000). However there is growing evidence for its role as an endocrine and behavior disruptor in frogs and salamanders. Exposure to extremely low levels (≥ 0.1 ppb) of atrazine in the lab produced hermaphroditism in *Xenopus laevis* larvae, and lowered plasma testosterone levels ten-fold (Hayes et al. 2002). Hayes et al. (2003) also showed similar effects on wild populations of Leopard Frogs (*Rana pipiens*) exposed to environmental atrazine levels of as low as 0.2 ppb. Species with aquatic larvae (such as *D. quadramaculatus*) or with an entirely aquatic life cycle (such as Hellbenders, *Cryptobranchus alleganiensis*) may be most susceptible to low level pesticide exposure (e.g., Berrill et al. 1998). Hellbenders appear to have declined substantially in the GSMNP since the 1950's (Nickerson et al. 2002), so there is considerable interest in identifying possible causal factors.

The two sites with the highest number of pesticides were the two most western sampling locations. Current-use and banned pesticides occurred at both of these sites, and levels were generally higher than at the other sites. The Foothills Parkway site is bordered to the east and west by private land, and much of this is cropland. Pesticide residues were absent only in the most eastward site in North Carolina (Kephart Prong). However, the overall geographic pattern was heterogeneous and did not clearly match our predictions. The remaining sites showed no consistent eastward decrease in number or level of agrichemical residues. They contained either

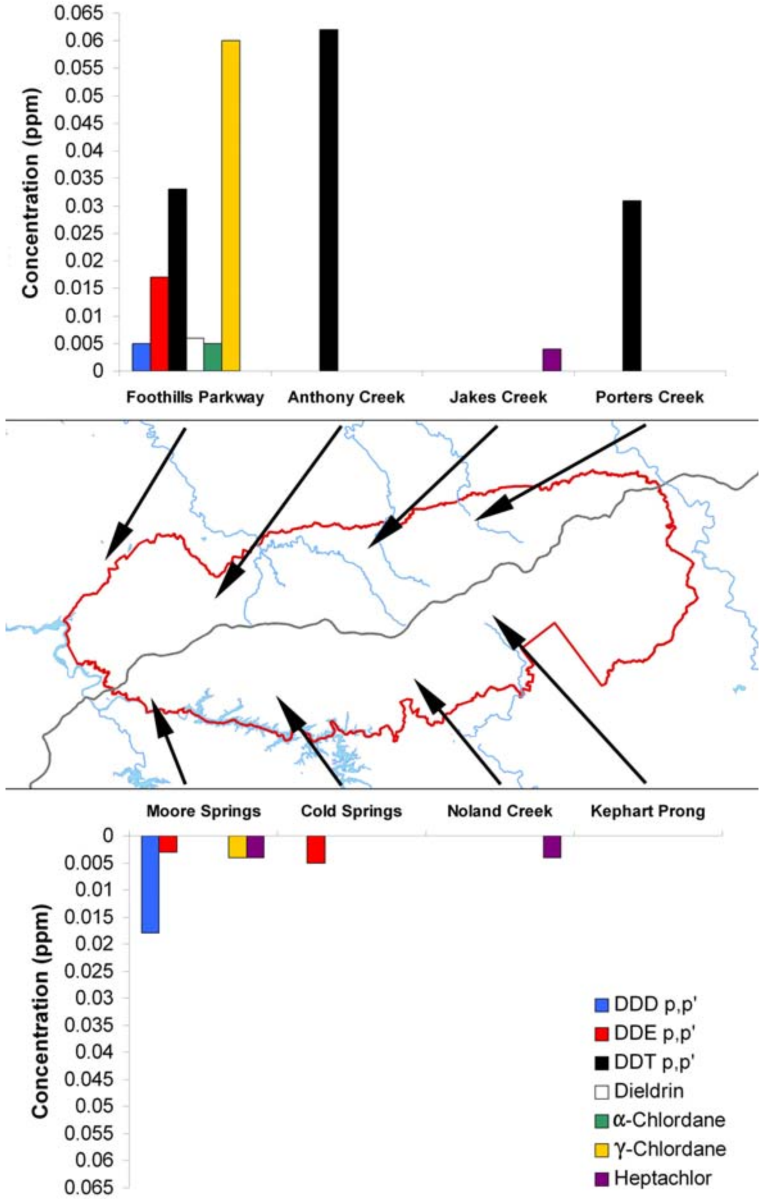

FIGURE 2.—Maximum concentrations of banned, historic-use pesticide residues identified in lipid tissue from the tail of *D. quadramaculatus* salamanders from transect sites in Tennessee and North Carolina, USA, during 2001 and 2003.

banned or current-use agrichemicals, but not both (except at Noland Creek). Although the prevailing summertime regional wind direction is from the southwest, there is considerable local variation in wind direction at many sites in the GSMNP (Fig. 3). The most significant wind directions at the Foothills Parkway site are WNW and SSE, and at the Moore Spring site the prevailing wind directions are westerly and easterly.

Rainfall tends to increase substantially with elevation (Fig. 3); however, there is likely to be considerable variation at a much smaller scale than can currently be resolved. Aspect and elevation possibly confounded our results because the environmental half-life of some agrichemicals can vary with environmental factors such as temperature and light intensity (e.g., Rocha and Walker 1995). As a result, patterns of agrichemical deposition and persistence are probably highly heterogeneous within the GSMNP and difficult to predict. Nevertheless, our study indicates that amphibians within the GSMNP are exposed to both historical and current use agrichemicals. These environmental stressors may therefore pose a significant

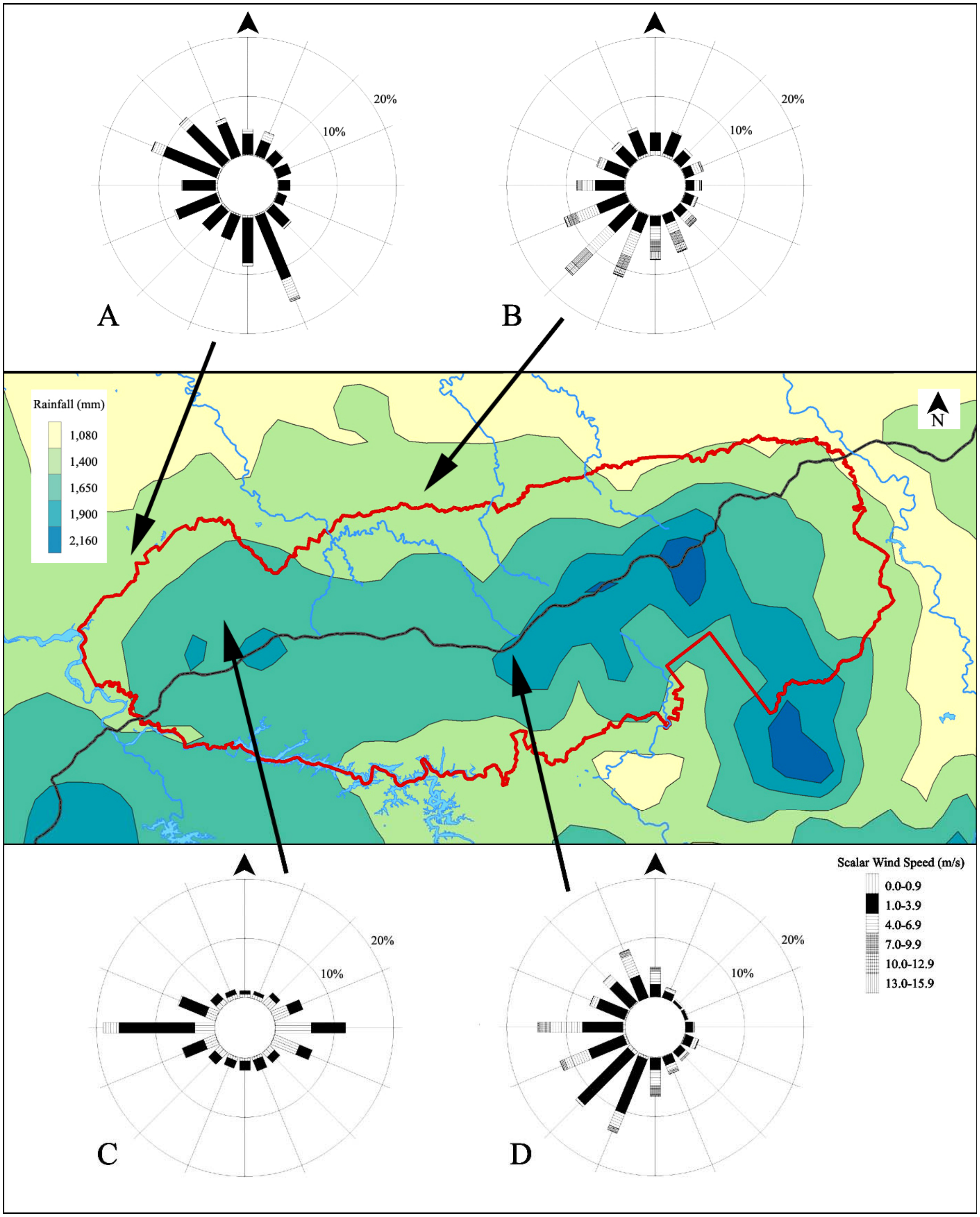

FIGURE 3.—Wind directions and precipitation in the Great Smoky Mountains National Park. The length of the bars on the wind roses show the percentage of time the wind blew from each direction in 2003. Wind velocity is indicated by the shading pattern of each bar. Arrows point to the location of monitoring stations. Wind data are from the National Park Service Gaseous Pollutant Monitoring Program Database, available from http://12.45.109.6/ (Accessed 13 March 2007).

risk to some amphibian species within the park, and we recommend further studies to identify and explain spatial and temporal patterns of agrichemical deposition within the GSMNP.

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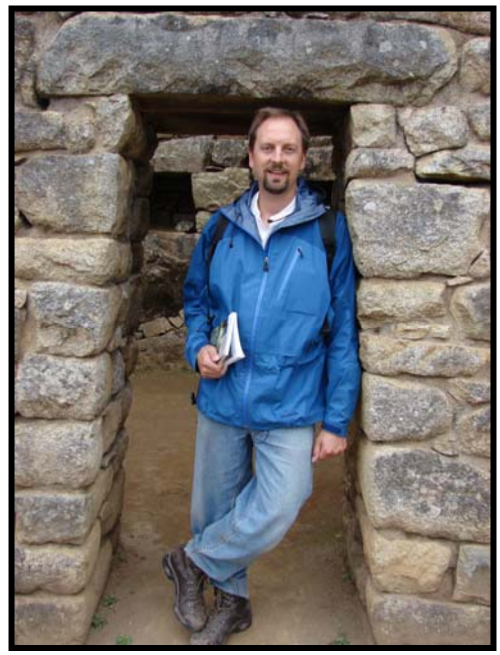

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