DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION 5

FY05 ENVIRONMENTAL CONTAMINANTS PROGRAM
Off Refuge Investigations Sub-Activity
FINAL REPORT

SALAMANDER ASSEMBLAGE SURVEY OF MERCURY AND SELENIUM CONTAMINATED HEADWATER SITES IN THE APPALACHIAN MOUNTAINS OF PENNSYLVANIA, VIRGINIA, AND WEST VIRGINIA

Project ID:5f37/20035002
(file name: FINALSALLY.DOC)

Submitted by:

Kathleen Patnode
Cindy Kane
Dan Ramsey
Jessica Rhodes
Brian Evans
Environmental Contaminants Specialists

For:

David Densmore
Pennsylvania Field Office Supervisor

Karen Mayne
Virginia Field Office Supervisor

August 1, 2005

ABSTRACT

Headwater streams comprise 60-75 percent of the total stream length and watershed area in the Mid-Atlantic region. Due to their diverse and complex life histories and abundance in the Mid-Atlantic Highlands, Stream Plethodontid salamanders are a potential biological endpoint to assess headwater impairment and degradation from contaminant exposure, especially where traditional species assemblages (macroinvertebrates, fishes) are poorly developed or absent. In this study, we conducted salamander assemblage surveys of headwater sites and determined contaminant exposure in the highlands of Pennsylvania, Virginia, and West Virginia to assess potential effects of inorganic contaminant exposure on the salamander community, and potential risk of selenium and mercury to upper trophic level predators. We conducted salamander surveys on 32 study areas and analyzed 182 salamander samples from 50 study areas. Mean concentrations exceeded the respective toxicity reference values selected for salamanders at multiple study areas for selenium, mercury, aluminum, and copper. Selenium concentrations in salamanders from study areas downstream of mining valley fills were significantly higher than study areas exposed to high air emissions or no reported emissions according to the USEPA Toxic Release Inventory. The proportions of reference, intermediate, and impaired salamander assemblages are significantly different ($p = 0.038$) between study areas where selenium exceeds the toxicity reference value and those that do not. The proportions of reference, intermediate, and impaired salamander assemblages are significantly different ($p = 0.005$) between study areas downstream of mining valley fills versus those exposed only to selenium air emissions deposition. Selenium exceeded toxicity reference values at 11 study areas for Louisiana waterthrush, while one study area was below the toxicity reference value for northern water shrew. Mercury toxicity reference values were exceeded at two study areas for waterthrush and at no study area for water shrew. Our data indicate that selenium exposure may be a factor affecting headwater biota downstream of mining valley fills. Our ecological risk assessment demonstrates that high-exposure risk scenarios with relevant receptors, particularly State-status species, should be considered in establishing aquatic selenium and mercury criteria that are protective of birds and mammals. Our data substantiate the need to couple salamander sampling with fish sampling to monitor ecological health in headwater streams and to protect their complex aquatic and aquatic-dependent animal communities.

PREFACE

This report summarizes salamander tissue analytical and population survey results performed for study areas located throughout Pennsylvania, West Virginia, and Virginia. Questions, comments, suggestions, and data requests related to this report are encouraged. Written inquiries should refer to Project ID:5f37/20035002 and be directed to:

Kathleen Patnode
U.S. Fish and Wildlife Service
c/o U.S. EPA
1060 Chapline St Suite 303
Wheeling WV 26003-2995
kathleen_patnode@fws.gov

ACKNOWLEDGEMENTS

Gian Rocco and technicians from Penn State Cooperative Wetlands Center of the Pennsylvania State University performed salamander assemblage surveys and habitat assessments for some of the study areas. Rocco also trained USFWS staff on the survey method and graciously permitted USFWS to use population data that were collected for Rocco and Brooks (2000) and Rocco *et al.* (2004). He also provided the formulae for applying the SPAR Index of Biotic Integrity to our habitat and salamander data, and assisted with interpretation of the scores. Without his body of research, this study would not have been possible.

Dr. Tom Pauley, Linh Phu, Michael Osbourn, and Dr. Mark Watson of Marshall University provided salamander population data for study areas in West Virginia that were surveyed for Pauley *et al.* 2004. Jeff Bailey of WVDEP provided habitat data for the study areas surveyed by Marshall University.

Bruce Pluta and Kathryn Davis of the U.S. Environmental Protection Agency assisted with the development of the ecological risk assessment conceptual site and food chain models. They were also instrumental in interpretation of the resulting HQs.

Robin Jung of USGS, Patuxent Wildlife Research Center provided input on study area selection and shared data from the long-term amphibian monitoring that she oversees. Ken Sturm at the USFWS Canaan Valley Wildlife Refuge collected salamanders for tissue samples from the refuge and provided habitat and population data that are part of the USGS study.

U.S. Department of Agriculture, Forest Service, George Washington and Jefferson National Forest, Warm Springs and Deerfield Ranger Districts, with particular thanks to Dawn Kirk, Fred Huber, Terry Flaherty, Sarah A. Davis. The Virginia Department of Game and Inland Fisheries at Hidden Lake Wildlife Management Area; Bill Wasserman; and the USFWS's Southwestern Virginia and Virginia Field offices: Kerry Linehan, Kristal McKelvey, Julia Harrison and Gale Heffinger.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS/ABBREVIATIONS

ARMI	Amphibian Research and Monitoring Initiative
HQ	Hazard Quotient = tissue concentration/LOED
HQ<1	Below potential risk level
HQ>1	Above potential risk level
IBI	Index of Biotic Integrity
LOED or LOEL	Lowest observed effect dose or level
mg/kg	milligrams per kilogram
NOED or NOEL	no observed effect dose or level
SPAR	Stream Plethodontid Assemblage Response
TRVs	toxicity reference values
TRI	USEPA Toxic Release Inventory
WAS	West Virginia Division of Environmental Protection Watershed Assessment Section

I. INTRODUCTION

A. Background and Justification

Small headwater streams are vital components of rivers. They are where water initially leaves the soil, enters the channel, and begins its journey downstream. As a result of this close association with the surrounding landscape, headwaters are a critical source of water, food, natural sediments, and nutrients for lower stream reaches (Gomi *et al.* 2002). They also represent areas of potentially high geological and biological diversity, providing habitat for numerous species and natural communities (Meyer and Wallace 2001).

Headwater streams comprise 60-75 percent of the total stream length and watershed area in the Mid-Atlantic region (Rocco *et al.* 2004). In this region, many headwater watersheds are heavily forested; a majority of watersheds have at least 60 percent forest cover (based on 7-digit USGS Hydrologic Unit Code boundaries) (Jones *et al.* 1997). Although these streams and their associated riparian areas play a major role in determining downstream water quality, and are vital habitat for terrestrial and aquatic species, relatively little effort has been made to include them in watershed pollution assessments. Encompassing large surface areas, Mid-Atlantic headwater forests receive a significant proportion of atmospheric pollutant deposition from both local and distant air emission (emission) sources. In addition, many Mid-Atlantic Highland headwaters overlay major coal deposits. The practice of "valley filling," or depositing mining overburden in headwater ravines, has physically affected salamander communities (Williams 2003). Water monitoring downstream of these valley fills has demonstrated that contaminants leach into headwater streams in some locations (Bryant *et al.* 2002). However, the impact of these contaminants in headwater streams in the Mid-Atlantic has not been thoroughly investigated.

Traditional methods of assessing aquatic ecosystem health using fish communities are often unsuitable for headwater streams, where flow or habitat often limits fish populations. A suitable ecological indicator is needed for forested headwaters that could provide a reliable expression of environmental stress or change that can help scientists, managers, and policymakers document trends, establish priorities, and target restoration activities. Stream Plethodontid salamanders are abundant and populations are stable and geographically widespread in the Northeast and Mid-Atlantic (Stuart *et al.* 2004). By virtue of their diverse and complex life histories, these salamanders are a potential biological endpoint to assess headwater impairment and degradation, especially where traditional species assemblages (macroinvertebrates, fishes) are poorly developed or absent. In this study, we conducted salamander assemblage surveys of headwater sites and determined contaminant exposure in the highlands of Pennsylvania, Virginia, and West Virginia to assess the potential effects of contaminant exposure on the salamander community, and the potential risk of bioaccumulative contaminants to upper trophic level predators.

B. Mercury

Emissions from coal-fired power plants are the largest identified contributor to atmospheric deposition of mercury in the United States (USEPA 1998). Mercury emissions are related to the age of the power plant, as well as the volume and source of coal combusted. Coal-fired power plants in Virginia, West Virginia, and Pennsylvania contribute nearly 17 percent of the 48 tons mercury/year emitted nationwide from coal-fired power plants and other mercury-emitting industries. Under the U.S. Environmental Protection Agency (USEPA) Clear Skies Initiative, mercury emissions would be reduced to a 26-ton annual cap by 2010 with a national trading program, and a 15-ton presumptive annual cap in 2018 (USEPA 2002a). Voluntary reduction encouraged under the Clear Skies Initiative will be undertaken while coal production from the Mid-Atlantic Highlands is expected to increase by 40 million tons and the contribution of coal combustion to electric power generation is likely to remain at 45 percent (USEPA 2002b). Details are lacking in the Clear Skies Initiative on expected mercury emission reductions in high deposition areas, and the biological impact reduction for this bioaccumulative substance. Existing mercury contamination and continued mercury deposition have ramifications for the Clean Water Act Section 303(d) Total Maximum Daily Load program, particularly in watersheds where mercury uptake to aquatic biota has already been documented (USEPA 2000a, Cherry *et al.* 2000).

The highest deposition rates occur in the Ohio River valley and northeastern United States due to the location of sources and climate (e.g., high humidity). High elevation areas receive significant mercury deposition that has resulted in fish consumption advisories in lakes, reservoirs and ponds (USEPA 1998). The relative contributions of local versus distant sources depend on numerous factors and remain a controversial issue. The USEPA does not consider atmospheric depositions to be a leading source of water quality impairment in streams and rivers. However, research in the headwaters of Lake Champlain (Rea *et al.* 1996) demonstrates that measurement of mercury concentrations in precipitation underestimates the deposition of mercury in forests. Throughfall water (i.e., precipitation falling on leaves and running off onto the forest floor) had twice the concentration of mercury than rainwater. In addition, mercury loading from litterfall (i.e., contaminated leaves depositing on the forest floor) was an order of magnitude greater than precipitation loading. Further study in the same area (Scherbatskoy *et al.* 1998) demonstrated that forests can capture and retain as much as 95 percent of the atmospheric mercury they receive. Their data indicate that in-stream organic matter may be a significant factor in mercury retention in headwater streams. In a USEPA-funded study in New York, Driscoll *et al.* (2001) have made similar observations on the contribution of litterfall to mercury on the forest floor and in leachate. Since leaf drop each fall contributes significant litter to headwater tributaries, it is reasonable to conclude that significant mercury loading occurs each year.

The chemical form of mercury influences both its uptake and toxicity. Mercury deposition occurs predominantly in the elemental form, while mercury bioaccumulation is most significant in the methylated form. Conversion of elemental to methylmercury occurs in anaerobic conditions, predominantly in sediments (Wiener *et al.* 2003). The primary route of exposure in vertebrates is through ingestion of contaminated prey. Methylmercury is readily absorbed from prey by the gastrointestinal tract lining and easily crosses biological membranes, such as the blood-brain barrier. Methylmercury impairs reproduction, development, and neurological function (Eisler 1987). Elemental and methylmercury have no known biological function in vertebrates (i.e., they are not micronutrients), and the difference between tolerable natural background concentrations and toxic exposures is exceptionally small (Eisler 1987).

C. Selenium

Selenium is another inorganic substance known to bioaccumulate (Hamilton 2002) and impair fish and wildlife health (Lemly 2002). As with mercury, the form of selenium affects both uptake by and toxicity to biota. Selenite is the predominant form of selenium in soils of humid regions such as those found in the eastern United States (Ohlendorf 1989). Besser *et al.* (1989) demonstrated that selenite and organic selenium compounds (e.g., Se-methionine) are far more bioaccumulative than selenate. Selenite uptake is higher in shallow waters, fine sediments, and high organic matter. These conditions exist in headwater streams, particularly those streams that receive organic matter loading from deciduous leaf drop. Selenite is also more acutely toxic than selenate (Canton 1999).

Selenium is emitted into the atmosphere by coal combustion and other chemical industry processes. It can also be released into the environment from coal mining operations that deposit overburden into valley fills, thereby increasing leaching potential. States receiving a high proportion of emissions from local and distant sources also include large areas of past and current coal mining. Surface water monitoring in West Virginia indicates that exceedances of selenium water quality criteria are closely related to placement of upstream valley fills (Bryant *et al.* 2002).

Selenium is a known micronutrient in vertebrates, but the range between dietary requirements and toxic levels is relatively narrow (Ohlendorf 1989). Selenium causes embryo mortality, deformities, growth impairment, infertility, and aberrant behaviors (Lemly 2002). The primary route of exposure is via ingestion of contaminated prey, although maternal transfer to eggs is a significant route for effects on embryos.

D. Other Inorganic Contaminants

Air emissions from coal burning and chemical processes are not limited to mercury and selenium. Depending on the particular industry, a suite of inorganic contaminants is commonly released to the air. USEPA's Toxic Release Inventory (TRI) clearly demonstrates the potential for mixtures of contaminants from local and regional sources to be deposited in Mid-Atlantic forested headwaters. Contaminants released in appreciable mass in Pennsylvania, West Virginia, and Virginia, in addition to mercury and selenium, include aluminum, arsenic, barium, cobalt, chromium, copper, lead, manganese, nickel, and zinc. Exposure to biota in headwater streams would vary with each contaminant. Risk to biota would, in turn, depend on the toxicity of each contaminant, as well as any interactions resulting from exposure to combinations of contaminants.

E. Plethodontid Salamanders

Plethodontidae is the most diverse family of salamanders in the world, with over 200 species. Thirty-four species of these lungless amphibians are found in Appalachian forests, giving this ecoregion the highest salamander diversity in the world. Pennsylvania has 15 species, West Virginia 27 species (including one federally-listed, threatened species), and Virginia 32 species (including one federally-listed, endangered species), with the greatest diversity occurring in the Mid-Atlantic Highlands. They thrive and reproduce in seeps, brooks, and small streams, sometimes occurring in extremely high densities. Life histories within this group are highly variable, and consist of aquatic and terrestrial egg-laying species with variable aquatic larval periods (8 months to 4.5 years). They are called lungless salamanders because they absorb oxygen through the skin and the lining of the mouth. Unlike species that breed in vernal pools, populations of most stream-dwelling salamanders tend to be remarkably stable over time.

In the Mid-Atlantic Highlands Stream Assessment (USEPA 2000b), contaminant exposure and higher trophic level health were not determined in 44 percent of first and second order streams due to the absence of fish. In headwater streams, several Plethodontid salamanders replace fish and become the dominant vertebrate predator. They also serve as prey for higher trophic level birds and mammals. In these environments, this group becomes the preferred, if not the only, vertebrate bioindicator for assessing stream health.

Benthic macroinvertebrates can be used to identify direct toxicity from contaminant exposure. However, this assessment endpoint is of minimal use for contaminants such as mercury and selenium which pose the greatest risk due to bioaccumulation and biomagnification. Commonly, concentrations of bioaccumulative contaminants will not exceed sediment criteria protective of aquatic invertebrates. Consequently, organisms survive, but also accumulate the contaminants, thus exposing upper trophic level species (e.g., fish and amphibians) to concentrations that have the potential to cause population-level effects (USEPA 2000b).

Salamander toxicity reference values for tissue are severely limited. Sufficient acute toxicity data are available to demonstrate that salamander sensitivity is comparable to that of commonly tested amphibians (i.e., *Xenopus, Rana, Bufo*) and fish. As with other amphibian and fish species, the egg and larval stages of salamanders appear to be the most sensitive life stages (Schuytema and Nebeker 1996).

Assessment of the salamander community may also be more efficient than macroinvertebrate assessments. The number of species of salamanders at multiple life stages is relatively small compared to the species of invertebrates. Identification for most can be completed in the field with minimal laboratory verification of voucher specimens. This field-based approach is an advantage over the extensive laboratory time needed to accurately identify macroinvertebrate taxa.

In response to global concerns about amphibian health and survival, Congress appropriated funds to the U.S. Geological Survey in 1999 to establish the Amphibian Research and Monitoring

Initiative (ARMI). Two major objectives of the ARMI are to determine the status and trends of amphibian populations on Department of the Interior lands, and determine causes of declines, malformations and diseases. These efforts have included salamander monitoring in two national parks in the Appalachian region (Jung *et al.* 2001). Recent USGS studies have examined potential impacts due to acid deposition, but metal exposure and effects have not been evaluated.

The Stream Plethodontid Assemblage Response (SPAR) Index of Biotic Integrity, developed under USEPA's Science to Achieve Results Program, was designed to evaluate the impacts of multiple stressors on salamander communities occupying headwaters and seeps in the USEPA's Mid-Atlantic Highlands Environmental Monitoring and Assessment Program Area. A pilot project conducted in 1997-98 in 14 headwaters in the Allegheny Plateau, Pennsylvania (Rocco and Brooks 2000), showed significant salamander community responses to various forms of stream impairment, specifically acid mine drainage, episodic acidification, and riparian corridor degradation. More recently, the Stream Salamander Index of Biotic Integrity, developed for Maryland, shows a remarkable ability to distinguish degraded from non-degraded streams (Southerland *et al. in prep.*).

Availability of an assessment tool for seeps and headwaters such as SPAR would dramatically improve the ability to quantify ecological risk from exposure to these bioaccumulative contaminants in these ecologically critical headwater streams. Documentation of impacts to salamanders (e.g., changes in community structure, life stages, and biomass) in these habitats would facilitate the development of measures to reduce exposure and protect or restore headwater communities. Appropriate protections for headwater streams may also prevent future downstream contamination by removing sources that could contribute to stream and river contaminant loading. We report on efforts to test the use of the SPAR Index of Biotic Integrity on seeps and headwater streams receiving mercury, selenium, and other inorganic contaminants from atmospheric deposition and valley fill leaching.

F. Study Objectives

1. Document the spatial distribution of selenium, mercury, and other inorganic contaminants in Plethodontid salamanders in headwater streams of Pennsylvania, West Virginia, and Virginia.

2. Use the Stream Plethodontid Assemblage Response (SPAR) Index of Biotic Integrity to assess the potential impacts of mercury, selenium, and other inorganic contaminants over a broad exposure range on salamander abundance, species diversity, and life stage presence in headwater streams

3. Perform an ecological risk assessment to identify watersheds where the bioaccumulation of mercury and selenium in salamanders results in tissue concentrations that exceed levels known to produce toxic effects in aquatic and terrestrial predators.

II. METHODS

A. Study Area Selection

We selected 50 study area watersheds within the Mid-Atlantic Highlands of Pennsylvania, Virginia, and West Virginia. Study area headwaters fell into one of four categories: 1) within 50 km of a coal-fired power plant with no known history of mining; 2) within 50 km of a coal-fired power plant with a history of mining; 3) more than 100 km from a coal-fired power plant with a history of mining; and 4) more than 100 km from a coal-fired power plant with no known history of mining. We selected study areas and transects within study areas to minimize terrestrial and aquatic habitat differences for parameters that influence stream salamanders such as acid mine drainage, and riparian corridor degradation (Rocco and Brooks 2000, Pauley *et al.* 2004). We targeted sites with documented mercury or selenium contamination along with sites suitable as regional reference areas. The USEPA Mid-Atlantic Highlands Assessment Habitat database, USGS National Water Quality Assessment database, Bryant *et al.* (2002), and State water quality databases were screened for all relevant watersheds in the Appalachian ecosystem. Investigators selected study areas in partnership with State natural resource agencies, Gian Rocco (Pennsylvania State University), Dr. Jung (USGS), Dr. Pauley (Marshall University, West Virginia), and the U.S. Forest Service based on evaluation of known media and fish contamination, mining operations, coal-fired power plant sources, and salamander community data.

B. Stream and Seep Salamander Surveys

We conducted salamander surveys within 32 of the 50 study areas according to the protocol of either Rocco and Brooks (2000) or Pauley *et al.* (2004). Riffle/run portions of each headwater stream were sampled along a 100-200 m reach. For seeps, one continuous 10 m reach was sampled. Within each reach, three (Rocco and Brooks 2000) or two (Pauley *et al.* 2004) 4-m^2 rectangular plots were thoroughly searched. Each plot encompassed the land-water interface of the stream within riffle/run habitat. We removed all rocks, logs, and debris within each plot and raked the substrate by hand to search for concealed animals. Adults and larvae were captured by hand or net until the entire plot had been searched. Sites surveyed using the methods of Pauley *et al.* (2004) also included two-pass sampling of a 15-m x 2-m transect, with 1 m on the bank and 1 m in the wet channel. Cover objects were lifted and any salamanders underneath were collected. Salamanders were identified by species and life stage. Data were recorded on size, weight, and incidence of abnormalities, by species and life stage.

Habitat assessments were either extracted from existing databases or conducted according to Ohio EPA (2001) or West Virginia Division of Environmental Protection Watershed Assessment Section (WAS) protocols. For study areas that were part of either the Mid-Atlantic Highlands Assessment or the Draft Programmatic Environmental Impact Statement on Mountaintop Mining/Valley Fills in Appalachia (USEPA 2003), water quality and physical habitat data were already available. For sites in West Virginia surveyed by Marshall University, data were collected on water quality, physical habitat, and land use using the WAS protocol. For all other sites, habitat data were collected for parameters listed in Table 1 based on Rocco and Brooks (2000).

To account for the potential effect of salamander collection and removal conducted prior to salamander community surveys, an adjustment factor was applied to species abundance numbers. For two sites surveyed post-collection, a species collected during sampling but not documented thereafter during community assemblage surveys were identified as such. Adjustment was based on a comparison of the number of individuals of a species collected within the 3000-foot collection reach with the species and associated numbers documented in the 300-foot survey reach. Species counts were adjusted for three sites, and one species each added to two sites. The IBI score increased slightly for only one of these five study areas; however, assemblage classifications did not change.

C. Sample Collection

We collected salamanders at all 50 study areas for tissue analysis. We limited collection to adults for species that were abundant or large enough to achieve tissue mass requirements with a small number of animals. This approach was warranted to minimize sampling impacts on the salamander communities. No State or federally listed endangered species were collected for analysis.

We euthanized, measured, weighed, and segregated salamanders by species and size. Within species and size classes, we formed composites as necessary to meet tissue mass requirements. We submitted frozen samples to a USFWS Patuxent Analytical Control Facility-approved laboratory.

To assess the contribution of leaf litter contamination to in-stream contaminant levels, we collected dominant leaf litter at all sites. Samples were also submitted to a USFWS Patuxent Analytical Control Facility-approved laboratory for inorganic analyses.

D. Contaminant Analysis

In 2003, we submitted 85 samples for mercury and selenium analyses and 42 samples for selenium, mercury, and routine metals analyses representing 29 study areas. Routine metals analyses were warranted to compensate for the absence of contaminant data on these salamander species, and to address potential confounding with mercury and selenium effects on population endpoints. In 2004, we submitted 55 new samples from 21 additional study areas.

Relevant detection limits for bioaccumulative substances were difficult to achieve using routine analytical techniques. In 2003, detection limits were highly variable and often exceeded relevant criteria. Similarly, deciduous leaves collected from the streambanks were analyzed, but no detectable concentrations were documented. Given the higher concentrations present in biota, investigators focused solely on analysis of salamander samples in 2004.

Analytical detection limits were improved by switching to the methods employed by Trace Element Research Laboratory (TERL) at Texas A&M University (College Station, Texas). All samples were freeze-dried and homogenized prior to digestion. Tissue digestion of freeze-dried, powdered tissue utilized nitric acid, hydrogen peroxide, and hydrochloric acid in a block digester. Following digestion, samples were diluted as necessary and analyzed for trace metals depending on analytical methods. Divalent mercury (Hg^{++}) in samples was reduced to the elemental state (Hg^0) by a strong reducing agent (stannous chloride). Mercury concentration in the sample was determined by cold vapor atomic absorption spectroscopy. Tissue digests were heated with HCl to convert Se(VI) to Se(IV) to facilitate hydride generation. Atomic fluorescence spectroscopy, one of the most sensitive methods currently available for inorganic analysis, was used to determine selenium concentrations. Silver, arsenic, cadmium, lead, and vanadium concentrations were determined using inductively coupled plasma-mass spectroscopy to achieve extremely low detection limits. All other inorganics were analyzed using inductively coupled plasma optical emission spectroscopy.

The duplicate testing of 15 samples from 2003 was used to assess the variability in analytical results between the 2003 and 2004 laboratories. The TERL methodology for selenium had lower detection limits and consistently higher concentrations were measured. Paired analytical results for selenium were used to derive a linear regression equation (Appendix A). This equation was applied to the 2003 selenium data to correct for the differences in methods between the two laboratories. These corrected values for selenium were used in all subsequent data analyses. Detection limits were also lower for mercury, but consistent differences were not observed. Therefore, no correction was made to 2003 mercury concentrations. All samples without detectable concentrations were evaluated at one half of the detection limit.

Quality assurance and quality control procedures were USFWS Patuxent Analytical Control Facility standards. Duplicates and spiked recovery results were within acceptable ranges. Contaminant concentrations were reported as milligrams per kilogram (mg/kg), wet weight. Analytes, tissue sample concentrations, and limits of detection are presented in Appendix B.

E. Data Analysis

1. Site classification:

We evaluated study areas based on the USEPA TRI for each county. This approach broadened the range of contaminants and their sources to include all reported air emissions in 2003. We documented physical evidence of mining during the habitat and salamander assessments, and verified our determinations by reviewing available water data and aerial imagery. We then categorized study areas as being beyond 50 km of any reported emissions, within 50 km of a county with low or high emissions (i.e.,first or second quantile of two quantiles), or downstream of mining valley fills (Table 2).

We used concentrations of inorganic contaminants in salamander samples to compute study area averages and maximums. We evaluated these data to determine if concentrations across all species should be pooled. We excluded data from northern spring salamanders (*G. porphyriticus*) as they are higher trophic level predators than the other species and were only available from a small number of study areas. We included data for the remaining species at each study area in the average and maximum determinations. We then compared the resulting study area averages and maximums relative to source classes.

2. SPAR IBI:

We applied the SPAR Index of Biotic Integrity that was developed for Mid-Atlantic habitats by Rocco *et al.* (2004). Rocco *et al.* used multiple discriminate analyses to develop a predictive model for the classification of sites into one of the three groups that differed with respect to geographic location and stream physical habitat. Groups 1 and 2 are steeper, have more boulder cover, and are cooler than Group 3 sites. Sites in Group 1 are northern Appalachian high-gradient streams, while Group 2 sites are southern Appalachian high-gradient streams. Group 3 consists of lower gradient sites that are geographically centered within the Mid-Atlantic region, and more widespread relative to the other groups.

We classified study areas into one of the three groups using four measurements (latitude, percent boulder cover, stream temperature, and gradient) obtained during stream habitat assessments (Table 1) or calculated from those data. Geographic coordinates for stream sites were determined from a map or by GPS on site. We estimated boulder cover by zigzag pebble count (Bevenger and King 1995) or as a percentage of each quadrant or transect. We determined stream gradient from 7.5-minute USGS topographic quadrangles, and we measured water temperature at each sampling plot with a thermometer. Using the coefficients and formula provided by Rocco *et al.*, we determined group membership for the 32 study areas surveyed.

The availability of a habitat-specific index of biotic integrity developed for salamanders in this ecoregion based on a large data set facilitated the evaluation of the small data set collected in this study. We derived IBI scores using the metric combinations that had the highest percentage of correctly classified sites in Rocco *et al.* (2004). Rocco *et al.* used Mann Whitney testing to identify the 11 best-performing metrics. Combinations of these metrics were then evaluated to determine their efficiencies at classifying salamander communities for all streams combined, degraded streams, and non-reference streams in a data set of over 130 streams. The resulting seven group-specific metrics, the IBI combinations, and their classification efficiencies are presented in Table 3. Rocco *et al.* used parametric, nonparametric, and multivariate analyses to derive habitat classification factors and habitat-specific metrics applicable to our study area. We used this habitat-specific IBI to score the 32 study areas for which salamander assemblage data were available. Although efficiencies for Group 2 sites were reduced (Table 3), we applied the group-specific IBIs as we had only one Virginia study area that classified as Group 2. IBI scores were then used to classify the salamander assemblages at each study area as reference, intermediate, or impaired.

3. Ecological risk:

We used literature-based toxicity data to classify potential risk to salamanders for each study area. Lowest observed effect dose (LOED) toxicity reference values (TRVs) for tissue concentrations (Table 4) were used to derive hazard quotients (HQs; HQ=tissue concentrations/LOED). If a LOED was not available, a level was estimated by multiplying the no observed effect dose (NOED) by a factor of ten. For chromium, a LOED was estimated by computing the geometric mean of the NOED and LD75 values. Toxicity data for salamanders were limited to cadmium. For other inorganics, we selected TRVs for other amphibians and fish. We determined HQs for each tissue sample for each contaminant. We then used average HQs for each study area to classify a study area as potentially at risk (HQ>1) or below known risk levels (HQ<1) for each contaminant. We assigned an overall HQ classification based on the highest HQ score for all contaminants. We then tallied the number of reference, intermediate, and impaired salamander assemblages based on HQ classification. We statistically compared the percentages of reference, intermediate and impaired salamander assemblages between low risk (HQ<1) and high risk (HQ>1) study areas using a Chi-square test.

Salamander contaminant concentrations were also used to assess the risk to predators inhabiting headwater streams. We selected the northern water shrew (*Sorex palustris*) as the mammalian ecological receptor. The water shrew is found in high elevation forests near mountain streams bordered by rocks, logs and over-hanging banks. The diet of the water shrew consists of aquatic insects, larvae, spiders, worms, small fish, fish eggs, amphibians, amphibian larvae (Jones and Birney 1988). We chose the Louisiana waterthrush (*Seiurus motacilla*) as the avian ecological receptor. The waterthrush lives in deciduous or mixed forests with rapid flowing streams. Their diet includes aquatic and terrestrial insects, small fish and small frogs (Cornell Laboratory of Ornithology). In highland headwaters where fish and frogs are often absent, it is reasonable to assume that aquatic salamanders would be included in their diet; Mulvihill (1999) has reported Louisiana waterthrush feeding immature terrestrial salamanders to their nestlings.

We derived the contaminant exposure value using a standard food chain model equation. We based the value on four parameters: food ingestion rate, contaminant in prey, body weight, and area use factor (Table 5). The northern water shrew body weight may range from 9 to 18 g (Burt and Grossenheider 1980, Pennsylvania Game Commission, Virginia Department of Game and Inland Fisheries). We used an average of the lowest body weights from these sources (10.3 g). According to Warrington (2001), due to their small size and high metabolic rate, the water shrew's food ingestion rate is equivalent to its own body weight. The Louisiana waterthrush weight averages 21.5 g (Environment Canada, Smithsonian National Zoological Park). The food ingestion rate formula for passerine birds (USEPA 1993) was used to derive an ingestion rate for the waterthrush. Media contaminant concentrations were not included because water exposures are highly transient and these headwaters do not accumulate significant amounts of fine sediment.

We compared the calculated contaminant exposures to NOAEL and LOAEL TRVs for the two ecological receptors (Table 5). For water shrew, we selected the mercury NOAEL and LOAEL values from a mink study with mortality, weight loss and ataxia endpoints (Wobeser *et al.* 1976). We based the selenium NOAEL and LOAEL values on potassium selenate in rats from Rosenfeld and Beath (1954) with a reproduction endpoint. These values were identified as the most appropriate TRVs by Oak Ridge National Laboratory (Sample *et al.* 1996). For the waterthrush, we selected the mercury NOAEL and LOAEL values from a mallard duck study with a reproduction endpoint (Heinz 1979), which was also preferred by Sample *et al.* (1996). We used selenium NOAEL and LOAEL values derived from EC_{10} and EC_{20} values for mallard egg hatchability as a function of selenium concentration in diet based on six studies (Hoffman *et al.* 2003). We used mallard body weight and food ingestion rate values from Wildlife Exposure Factors Handbook (USEPA 1993) to convert the mallard EC values to TRVs.

III. RESULTS

We collected a total of 779 animals for 182 samples (Table 6) from 50 study areas (Figure 1). Species collected were *Desmognathus fuscus, D. monticola, D. ochrophaeus, Eurycea bislineata, Gyrinophilus porphyriticus, Plethodon cinereus,* and *E. longicauda.* Thirty one animals (11 *D. monticola* , 15 *D. fuscus, and five G. porphyriticus)* were large enough to analyze without compositing. Average concentrations of selenium varied between species which likely reflects the duration of the obligate aquatic phase and dietary preferences (Figure 2). Selenium concentrations in northern spring salamander (*G. porphyriticus*) were consistently higher, which is likely due to their higher trophic level position and extended life spans. One terrestrial salamander sample (*P. cinereus)* was analyzed for comparison to species with an extended obligate aquatic life stage. Concentrations of selenium in this terrestrial salamander sample were lower than those in *E. bislineata* and *D. fuscus* of similar size from the same study area.

We computed average sample concentrations and salamander HQs for all inorganic contaminants for each study area. Selenium concentrations ranged from 1.06 to 14.32 mg/kg dry weight (dw), while mercury values were 0.031 to 0.36 mg/kg wet weight (ww) (Table 7). HQs ranged from 0.1 to 3.6 for selenium and 0.0 to 1.1 for mercury (Table 7). Average concentrations of selenium were above the respective TRVs for 16 study areas. Average mercury HQs were not greater than one at the two study areas, PA522 and PA544, where single samples exceeded the mercury TRV. Aluminum concentrations ranged from 0.01 to 550 mg/kg ww, while the range of copper concentrations was 0.01 to 18.42 mg/kg ww. Average HQs of greater than or equal to one were observed for aluminum (13 study areas) and copper (five study areas). Mean concentrations of all other inorganic contaminants were below their respective TRVs. Analytical results for samples for all analytes are presented Appendix B.

We classified study areas by proximity to potential sources of inorganic contaminants (Table 2). Spatial distribution of selenium concentrations relative to potential sources are presented in Figures 3 and 4. Similar data are presented for mercury in Figures 5 and 6, aluminum in Figure 7, and copper in Figure 8. Concentration categories in all six figures are based on salamander HQs; low and medium categories are equivalent to HQ less than one and high category is HQ greater than one. A comparison of salamander selenium concentrations between source classes is presented in Figure 9. Analysis of variance (ANOVA) indicated that both average and maximum selenium concentrations in salamanders from study areas downstream of mining valley fills were significantly higher than those with elevated or no reported emissions. We found no difference between average or maximum selenium concentrations or salamander assemblages in study areas with high emissions or no reported emissions.

We used SPAR IBI calculations to derive scores and classifications for salamander assemblages in 32 study areas (Table 8). Ten study areas were categorized as having salamander assemblages comparable to reference conditions, four as being intermediate, and 18 as being impaired. The proportions of reference, intermediate, and impaired assemblages by HQ are depicted in Figure 10. Chi-square analysis indicated that the proportions are significantly different ($p = 0.038$) between study areas with HQ less than one and those equal to or greater than one. The proportions of reference, intermediate, and impaired assemblages by potential source class are presented in Figure 11. Chi-square analysis indicated that the proportions are significantly different ($p = 0.005$) between study areas downstream of mining valley fills versus those exposed only to emissions deposition.

We also evaluated selenium and mercury risks to avian and mammalian predators. Selenium LOAEL HQs ranged from 0.10 to 3.61 in the waterthrush and 0.83 to 29.23 in the water shrew. Selenium HQs were greater than or equal to one at 11 study areas for the waterthrush (Figure 12), while only one HQ was less than one for the water shrew (Figure 13). Risk calculations for water shrew are not directly applicable in the southwest counties of West Virginia since this area is not documented to be within the species range. Potential risks from mercury exposure were considerably lower. LOAEL HQs for all 50 study areas were less than one for both waterthrush and water shrew. HQs for NOAELs ranged from 0.17 to 2.17 for the waterthrush and 0.06 to 0.74 for the water shrew. Mercury NOAEL HQs were greater than or equal to one at only two study areas for the waterthrush (Figure 14), and at no study areas for the water shrew (Figure 15).

V. DISCUSSION

A. Selenium

This study documented selenium exposure and effects on salamander assemblages in Mid-Atlantic Highland headwaters, and demonstrated the effectiveness of salamanders as a biomonitoring tool. We found impaired salamander assemblages in study areas where selenium concentrations in tissues exceeded the TRV. Where water concentrations [as available from Bryant *et al.* (2002) or WVDEP (unpublished water quality monitoring data)] were near or above the water quality criterion of five μg/L, salamander HQs were greater than one, with only two exceptions (one area where only three animals were found, and one where selenium exceedances had only been occurring for six months). Calculations show likely risk to salamanders at 15 study areas where the average HQ exceeded one and potential risk to salamanders at an additional ten study areas where at least one sample had an HQ greater than one. Of the 15 study areas with HQ greater than one, 12 were below valley fills and three were near counties with elevated selenium emissions. For all 25 study areas, 14 were downstream of mining valley fills, four were near counties with elevated selenium emissions, and six were in areas where no selenium emissions were reported. We found that salamander assemblages were more likely to be impaired downstream of valley fills than in other locations. We also found an elevated risk to waterthrush in eight study areas downstream of valley fills. Risk estimates are not available for a relevant mammalian receptor in southwest West Virginia. These data indicate that selenium exposure may be a factor affecting headwater biota downstream of mining valley fills.

Selenium is only one of several chemical parameters that may be elevated in streams below valley fills (Bryant *et al.* 2002). Sulfate, hardness, manganese, conductivity, and alkalinity increases ranged from 7.5- to 42-fold over reference conditions, with selenium increasing by 7.8-fold. Selenium is unique among these other parameters in that it is absorbed by and bioaccumulates in aquatic biota. We documented elevated selenium in Plethodontid salamanders downstream of valley fills, and reduced assemblages in a large proportion (nearly 60 percent) of study areas with elevated selenium in whole body samples. However, it is possible that other factors associated with valley fills are responsible for the observed effects on the salamander assemblages. Green *et al.* (2000) demonstrated that benthic macroinvertebrate communities were depressed below valley fills where conductivity was elevated. In contrast, Pauley *et al.* (2004) found that of West Virginia salamander species, only *E. cirrigera* was negatively affected by conductivities as high as 2900 Umhos/cm. The impairment of salamander assemblages could be the result of reduced prey abundance as macroinvertebrate communities were depressed. Williams (2003) concluded that salamander abundance is reduced downstream of valley fills, and associated this effect with a shift from rock to fine sediment substrate. In contrast, Green *et al.* (2000) found no difference in sediment deposition or embeddedness between valley fill and reference streams. Identification of the cause(s) of the observed effects in this study will require the derivation of salamander-specific TRVs in media and, where applicable, in tissues via laboratory testing for the chemical parameters associated with valley fills.

We also documented elevated risk to salamander communities at four study areas within and seven beyond 50 km of selenium emission sources, while eight study areas within 50 km of selenium emissions did not have elevated risk. We found no statistical difference in salamander selenium concentrations or salamander assemblages between study areas with emissions and those where none have been reported. Factors controlling selenium transport, wet and dry deposition, and bioavailability were not examined in this study, but may differ between the no risk and elevated risk study areas. In addition, the USEPA TRI data are reported on a wide spatial scale and do not distinguish between documented zero emissions and failure to report. This shortcoming may have resulted in study areas being under-classified for selenium emissions. Our data indicate that localized monitoring may be the only accurate method of identifying locations where selenium exposure is high enough to pose risks to headwater biota. Based on the correspondence between water and tissue selenium concentrations in our study, we recommend that surface water data be reviewed to identify first and second order streams where selenium from atmospheric deposition may be degrading local water quality. In these streams, headwater biota should be tested to evaluate bioaccumulation and risk.

Even in the absence of salamander-specific TRVs, selenium is a valuable indicator since it can be measured in biological tissues. Tissue concentrations integrate exposures over time compared to water samples. They also eliminate the need to estimate exposure based on water chemistry because they measure only bioavailable selenium. Most importantly, they provide relevant doses for a bioaccumulative contaminant. It is for these reasons that USEPA has proposed fish tissue-based selenium criteria. We advocate that USEPA and State regulatory agencies couple salamander sampling with fish sampling in their approach to monitoring ecological health to address the extensive proportion of watersheds that have no fish, but support complex aquatic and aquatic-dependent communities.

We developed ecological risk assessments for higher trophic levels based on the water shrew and the waterthrush. We found potential risk to water shrew at all study areas near emissions, and all but one study area with no reported emissions, while waterthrush HQs greater than one occurred at two study areas near emissions, and one with no reported emissions. We have demonstrated that salamanders, which feed primarily on macroinvertebrates, are contaminated. It is reasonable to assume that macroinvertebrates are their primary source of selenium exposure. Water shrew and waterthrush, which consume macroinvertebrates, salamanders, and other aquatic biota, will be exposed in nearly 100 percent of their prey. We used these protective exposure parameters to broadly encompass locations in need of further evaluation in the Mid-Atlantic Highlands. Potential risk to the water shrew is of concern given their dependence on high water quality streams and their population status in West Virginia (imperiled), Pennsylvania (protected), and Virginia (imperiled). The waterthrush is an ideal indicator since it is the only obligate avian species of headwater ecosystems in the eastern United States (Brooks *et al.* 1998). Our conceptual site model results in a significantly greater exposure than for species that have larger home ranges, feed at lower trophic levels, or have a broader diet. The model generates a safe prey concentration of approximately 1 mg/kg dw in contrast to 7 mg/kg dw proposed by Lemly (2002) and 4 mg/kg dw advocated in U.S. Fish and Wildlife Service comments (USFWS 2005) on USEPA's 2004 draft tissue-based selenium criterion. While we recognize that the chosen receptors do not exist in all locations or habitats receiving selenium contamination, we recommend that similar, high-exposure risk scenarios with relevant receptors be considered in establishing selenium criteria that are protective of birds and mammals.

B. Mercury

We also documented mercury exposure in salamanders, although average mercury concentrations in salamanders did not exceed the fish TRV at any study areas. However, individual samples had HQs greater than one at two study areas in Pennsylvania. We calculated NOAEL HQs greater than one for waterthrush for these two, and one additional study area. All three study areas were near mercury emission sources. With emissions and transport of mercury well documented in the Mid-Atlantic, we expected risk to be higher and more widespread. It is possible that salamander mercury concentrations are limited by the factors controlling wet and dry deposition, and the bioavailability of mercury.

The deposition of airborne mercury is influenced by air flow pattern, land form elevation and orientation, and precipitation rate (USEPA 1998). Our study areas were located primarily in forested headwaters at elevations likely to receive significant deposition. The two study areas with mercury salamander samples exceeding TRVs were located at elevations of 1390 feet and

1140 feet. The former was on a high elevation plateau. The latter was in a valley on the west side of a southwest to northeast-running ridge. Mercury deposition could be elevated in these areas due to local topography and orientation. Further examination of regional wind patterns and local precipitation rates, as well as the addition of dry deposition monitoring will be necessary to understand the factors that influence transport of mercury into forested headwaters.

In headwater streams, the rate of methylation is the primary factor controlling mercury bioavailability. Methylation is favored in anaerobic, acidic conditions with fluctuating water levels found in wetlands (Wiener *et al.* 2003). One of the study areas with elevated mercury has significant floodplain wetlands located upstream, which may provide suitable conditions for methylation. The presence of wetlands upstream was atypical of our study areas. In typical Mid-Atlantic headwater streams, methylating conditions may also occur in intermittent stream pools with heavy leaf litter that receive acidic precipitation. As we selected streams with near neutral pH to avoid the known effects of acid mine drainage on salamanders (Rocco and Brooks 2000), it is not likely that conditions in our study areas favored methylation. Additional study areas with upstream wetlands and/or reduced pH without acid mine drainage should be sampled to determine if higher salamander mercury concentrations are more prevalent under these conditions.

C. Other Inorganic Contaminants

We documented detectable concentrations of cadmium, iron, nickel, zinc, barium, lead, aluminum, copper, arsenic and chromium in salamander tissues. Only aluminum and copper were present at levels exceeding TRVs. However, only the cadmium TRV was based on salamander testing. It is possible that salamanders are more sensitive than the surrogate receptors for which TRVs were available. As these contaminants have relatively low bioaccumulation potential, they were not evaluated in the risk assessment model for birds and mammals. Aluminum and copper were not found to be associated with valley fills (Bryant *et al.* 2002). The USEPA TRI data indicate that aluminum is not reported to be released by many industries in the Mid-Atlantic Highlands. Copper releases are far more ubiquitous, but numerous factors could reduce copper transport to and bioavailability in headwater streams. As additional tissue-based TRVs for amphibians become available, the data collected in this study should be reevaluated to improve the assessment of risk to Plethodontid salamanders due to other inorganic contaminants in headwater streams.

VI. MANAGEMENT RECOMMENDATIONS

The results of this study lead us to make the following management recommendations:

1. Advocate that USEPA and State regulatory agencies combined salamander sampling and fish sampling in their approach to monitoring ecological health in watersheds that do not have fish, but nevertheless support complex aquatic and aquatic-dependent communities.

2. Recommend that high-exposure risk scenarios with relevant ecological receptors, particularly State-status species, be considered in establishing selenium and mercury criteria that are protective of birds and mammals.

3. Support the identification of the cause(s) of the observed effects on salamander assemblages via laboratory testing to derive amphibian, particularly salamander, toxicity reference values for ubiquitous contaminants.

4. Advocate assessment of the potential for release of selenium to headwaters within the Mid-Atlantic Highlands to inform both permitting and Clean Water Act 303(d) TMDL and 305(b) assessment processes.

5. Obtain and conduct reviews of surface water data to identify first and second order streams where selenium and mercury may be degrading local water quality. Advocate that States test headwater biota in identified streams to determine bioaccumulation and risk to aquatic and aquatic-dependent biota.

6. Encourage Fish and Wildlife Service Environmental Contaminant specialists to work with peers in relevant State and Federal agencies (e.g., State environmental agencies, NOAA, USGS) to examine regional wind patterns and local precipitation rates to understand the factors that influence transport and deposition of selenium and mercury into forested headwaters.

7. Advocate the addition of contaminant dry deposition monitoring by State and Federal scientific and regulatory agencies to more completely understand sources of exposure for headwater biota.

8. Support the testing of additional study areas with upstream wetlands and/or reduced pH, but without acid mine drainage inputs, to determine if higher salamander selenium and mercury concentrations are documented, and thus if there is a higher risk to aquatic and aquatic-dependent biota.

VII. REFERENCES

Besser, J.M., J.N. Huckins, E.E. Little, and T.W. LaPoint. 1989. Distribution and bioaccumulation of selenium in aquatic microcosms. Environ. Pollut. 62:1-12.

Bevenger, G.S. and R. King. 1995 A pebble count procedure for assessing watershed cumulative effects. Res. Pap. RM-RP-319. US Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO. 17 p.

Brooks, R.P., T.J. O'Connell, D.H. Wardrop, and L.E. Jackson. 1998. Towards a regional index of biological integrity: The example of forested riparian ecosystems. Environ. Monitor. Assess. 51: 131-143.

Bryant, G., S. McPhilliamy, and H. Childers. 2002. Final report: A survey of the water quality of streams in the primary region of mountaintop/valley fill coal mining, October 1999 to January 2001. USEPA, Wheeling, WV.

Burt, W.H. and R.P. Grossenheider. 1980. A Field Guide to the Mammals. Houghton Mifflin Co., Boston, MA.

Canton, S.P. 1999. Acute aquatic life criteria for selenium. Toxicol. Environ. Chem. 18:1425-1432.

Cherry, D.S., R.J. Currie, and D.J. Soucek. 2000. Review of the global adverse environmental impacts to groundwater and aquatic ecosystems from coal combustion wastes. A Report to Hoosier Environmental Council and Citizens Advisory Coal Council. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Cornell Lab of Ornithology. Birds in Forested Landscapes – Louisiana Waterthrush. http://www.birds.cornell.edu/bfl/speciesaccts/louwat.html. Accessed 2005 July 25.

Driscoll, C.T., J. Yavitt, R. Newton, and R. Munson. 2001. Chemical and biological control of mercury cycling in upland, wetland, and lake ecosystems in the Northeastern United States. Progress Report to USEPA National Center for Environmental Research. http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/253/report/2001.

Eisler, R. 1987. Mercury Hazards to Fish, Wildlife and Invertebrates: A Synoptic Review. USFWS Biological Report No. 85(1.10) 90 pp.

Environment Canada. 2002 Dec. Louisiana Waterthrush. http://wildspace.ec.gc.ca/life.cfm?ID=LOWA&Lang=e. Accessed 2005 July 25.

Fjeld, E., T.O. Haugen, L.A. Vollestad. 1998. Permanent impairment in the feeding behavior of grayling (*Thymallus thymallus*) exposed to methylmercury during embryogenesis. Sci. Total Environ. 213:247-254.

Gillilland, C.D., C.L. Summer, M.G. Gillilland, K. Kannan, D.K. Villeneuve, K.K. Coady, P. Muzzall, C. Mehne, and J.P Giesy. 2001. Organochlorine insecticides, polychlorinated biphenyls, and metals in water, sediment, and green frogs from southwestern Michigan. Chemosphere 44:327-339.

Green, J., M. Passmore, and H. Childers. 2000. A survey of the condition of streams in the primary region of mountaintop mining/valley fill coal mining. U.S. Environmental Protection Agency, Wheeling, WV.

Hamilton, S.J. 2002. Rationale for a tissue-based selenium criterion for aquatic life. Aquat. Toxicol. 57:85-100.

Heinz, G.H. 1979. Methyl mercury: reproductive and behavioral effects on three generations of mallard ducks. J. Wildl. Mgmt. 43: 394-401.

Jones, K. B., *et al.* 1997. An ecological assessment of the United States mid-Atlantic region: A landscape atlas. EPA/600/R-97/130. U.S. Environmental Protection Agency, Office of Research and Development. Washington, DC. 105 pp.

Jones, J. K., Jr. and E.C. Birney. 1988. Handbook of Mammals of the North-Central States. University of Minnesota Press. Minneapolis, MN.

Jung, R.E., K.C. Rice, C.K. Dodd, Jr., W.B. Hughes. 2001. Amphibian Research and Monitoring Initiative (ARMI) in the Appalachian Region. USGS Appalachian Region Integrated Science Workshop, Oct 22-26, 2001, Gatlinburg, TN.

Lemly, A.D. 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. Aquatic Toxicol. 57:39-49.

McGeachy S.M., D.G. Dixon. 1990. Effect of temperature on the chronic toxicity of arsenate to rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 47:2228-2234.

Mulvihill, R.S. 1999. Effects of stream acidification on the breeding biology of an obligate riparian songbird, the Louisiana waterthrush (*Seiurus motacilla*). Pp. 51-61 *in* Sharpe, W.E, and J.R. Drohan, (*eds*.) The effects of acidic deposition on aquatic ecosystems in Pennsylvania. Proceedings of the 1998 PA Acidic Deposition Conference, Vol. II. Environmental Resources Research Institute, University Park, PA.

Nebeker AV, G.S. Schuytema, and S.L. Ott. 1995. Effects of cadmium on growth and bioaccumulation in the northwestern salamander *Ambystoma gracile***.** Arch. Environ. Contam. Toxicol. 29:492-499.

Ohio EPA. 2001. Field evaluation manual for Ohio's primary headwater habitat streams. Ohio EPA, Division of Surface Water, Columbus, OH.

Ohlendorf, H.M. 2003. Ecotoxicology of Selenium. Pp. 465-500 *in* Hoffman, D.J. *et al.* (*eds*.) Handbook of Ecotoxicology. Lewis Publishers. Boca Raton, FL.

Ohlendorf, H.M. 1989. Bioaccumulation and effects of selenium in wildlife. Selenium in Agriculture and the Environment, SSA Special Publication No. 23. Madison, WI.

Pauley, T.K., L.D. Phu, M.S. Osbourn, M.B. Watson. 2004. Streamside salamanders as indicators of headwater stream health in West Virginia: A final report to the US EPA and the USGS on a two-year stream salamander study. Marshall University, Charleston, WV.

Pennsylvania Game Commission. 2004 Feb. Wildlife Notes, Shrews. http://www.pgc.state.pa.us/pgc/cwp/view.asp?a=458&q=150573 Accessed 2005 July 25.

Rea, A.W., G.J. Keeler, and T. Scherbatskoy. 1996. The deposition of mercury in throughfall and litterfall in the Lake Champlain watershed: a short-term study. Atmos. Environ. 30:3257-3263.

Rocco, G.L. R.P. Brooks, and J.T. Hite. 2004. Stream Plethodontid assemblage response (SPAR) index: development, application, and verification in the MAHA. Penn State Cooperative Wetlands Center, Pennsylvania State University Report No. 2004-1 prepared for US EPA Star Grant Program.

Rocco, G.L. and R.P. Brooks. 2000. Abundance and distribution of a stream Plethodontid salamander assemblage in 14 ecologically dissimilar watersheds in the Pennsylvania Central Appalachians. Report No. 2000-4. Pennsylvania State University, University Park, PA.

Rosenfeld and Beath. 1954. Effect of selenium on reproduction in rats. Proceedings of the Society for Experimental Biology and Medicine. 87:295-297

Sample, B.E., D.M. Opresko, G.W. Suter. 1996. Toxicological Benchmarks for Wildlife: 1996 Revision. ES/ER/TM-86/R3. Oak Ridge National Laboratory, Oak Ridge, TN.

Scherbatskoy, T., J.B. Shanley, and G.J. Keeler. 1998. Factors controlling mercury transport in an upland forested catchment. Water, Air and Soil Pollut. 105:427-438.

Schuytema, G.S. and A.V. Nebeker. 1996. Amphibian Toxicity Data for Water Quality Criteria Chemicals. USEPA NHEERL, Corvallis, OR. EPA 600-R-96-124.

Smithsonian National Zoological Park. Migratory Bird Center, Life History, Louisiana Waterthrush. http://nationalzoo.si.edu/ConservationAndScience/MigratoryBirds/Life_History/default.cfm?id=134. Accessed 2005 July 25.

Stuart, S.N., J.S. Chanson, N.A. Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, R.W. Waller. 2004. Status and Trends of Amphibian Declines and Extinctions Worldwide. Sci. 306:1783-1786.

USEPA. 2002a. Detailed projected outputs of IPM for Clear Skies and Base Case. http://www.epa.gov/airmarkets/epa-ipm/results.html. Accessed 2003.

USEPA. 2002b. Coal and the Clear Skies Initiative. http://www.epa.gov/clearskies. Accessed 2003.

USEPA. 2000a. The Quality of Our Nation's Water B A summary of the National Water Quality Inventory: 1998 Report to Congress. Washington, DC. EPA 841-S-00-001.

USEPA. 2000b. Mid-Atlantic Highlands Stream Assessment. USEPA Region III, Philadelphia, PA. EPA 903-R-00-015.

USEPA. 1998. Utility Air Toxics Report to Congress. http://www.epa.gov/mercury/report.htm Accessed 2003.

USEPA. 1993. Wildlife Exposure Factors Handbook. Office of Health and Environmental Assessment, Office of Research and Development, Washington, DC. EPA/600/R-93/187a.

USFWS. 2005. Letter from Everett Wilson to Steven Johnson in response to the U.S. Environmental Protection Agency's (EPA) request for scientific information, data, and views pertaining to the "*Draft Aquatic Life Criteria Document for Selenium*" (Federal Register 69(242): 75541-75546; December 17, 2004).

Van De Putte, L., J. Lubbers, Z. Kolar. 1981. Effect of pH on Uptake, Tissue Distribution and Retention of Hexavalent Chromium in Rainbow Trout (*Salmo gairdneri*). Aquat. Toxicol. 1:3-18.

Virginia Department of Game and Inland Fisheries. Virginia Wildlife Information System. Water Shrew. http://www.dgif.state.va.us/wildlife/species/display.asp?id=050009. Accessed 2005 July 25.

Warrington, Patrick D. 2001 Sept. Animal Weights and their Food and Water Requirements. Water Management Branch, Environment and Resource Division, Ministry of Environment, Lands and Parks, Government of British Columbia. http://wlapwww.gov.bc.ca/wat/wq/reference/foodandwater.html#northamer. Accessed 2005 July 25.

Weiner, J.G., D.P. Krabbenhoft, G.H. Heinz, and A.M. Scheuhammer. 2003. Ecotoxicology of Mercury. Pp. 409-263 in Hoffman, D.J. *et al.* (*eds.*) Handbook of Ecotoxicology. Lewis Publishers, Boca Raton, FL.

West Virginia Wildlife Magazine. Wildlife Diversity Notebook: The Northern Water Shrew. http://www.wvdnr.gov/wildlife/magazine/Current/wildlife_diversity_notebook.shtm. Accessed 2005 July 25.

Williams, J.M. Impacts on terrestrial and streamside herpetofauna by mountaintop removal mining in southern West Virginia. PhD Dissertation, West Virginia University, 2003. Morgantown, WV. http://etd.wvu.edu/templates/showETD.cfm?recnum=3231. Accessed 2005 July 25.

Wobeser, G., N.O. Nielson, and B. Schiefer. 1976. Mercury and mink II. Experimental methyl mercury intoxication. Can. J. Comp. Med. Pp. 34-45.

Table 1. Summary of physical habitat parameters collected for *Stream Plethodontid Assemblage Response* Index from study areas in Pennsylvania, West Virginia, and Virginia, 2003 - 2005 (Rocco and Brooks 2000).

Survey reach location geographic coordinates (using GPS or from maps)
Stream gradient (m/1000 m) using GPS coordinates and TopoScout (MAPTECH, www.maptech.com) or equivalent from center of survey reach
Maximum pool depth (cm) within survey plots
Wetted channel width (m) within survey plots
Stream and ambient air temperature (° C)
Riparian Zone Survey plot percent canopy cover Survey reach land cover (dominant trees, understory cover)
Survey Plot Substrate cobble cover, moss cover, bank vegetation
Survey reach pebble count (Bevenger and King 1995)

Table 2. Classification of study areas by proximity to potential contaminant sources.

SITE ID	Se Source	Hg Source	Al Source	Cu Source	Source CLASS*
PA 035	0	2	2	2	2
PA 082	1	2	2	2	2
PA 410	2	2	0	2	2
PA 411	0	2	2	2	2
PA 412	2	2	0	2	2
PA 413	1	2	0	2	2
PA 522	0	2	2	2	2
PA 544	0	0	0	1	1
PA 771	2	2	2	2	2
PA 787	0	0	0	1	1
PA 848	0	2	2	2	2
VA 508	0	1	0	2	2
VA 554	0	1	0	2	2
VA 990	0	2	2	2	2
VA 991	3	2	2	2	3
VA 992	3	2	2	2	3
VA 993	3	0	0	0	3
VA 994	3	2	2	2	3
VA 995	0	2	2	2	2
VA 996	0	1	0	2	2
VA 997	0	1	0	2	2
VA 998	0	2	2	2	2
VA 999	3	1	2	2	3
WV 018	3	2	0	2	3
WV 025	3	2	0	1	3
WV 039	0	2	0	1	2
WV 085	3	2	0	1	3
WV 088	3	2	0	1	3
WV 090	3	2	0	1	3
WV 092	0	2	0	1	2
WV 093	0	2	0	2	2
WV 504	2	2	2	2	2
WV 505	2	2	0	1	2
WV 982	2	2	0	2	2
WV 983	2	2	0	2	2
WV 984	2	2	0	2	2
WV 985	2	2	0	2	2
WV 986	3	2	0	1	3
WV 987	3	2	0	0	3
WV 989	0	2	0	0	2
WV 990	0	2	0	0	2
WV 991	3	2	0	0	3
WV 992	0	2	0	2	2
WV 993	3	2	0	0	3
WV 994	2	2	0	1	2
WV 995	3	2	0	0	3
WV 996	0	2	0	1	2
WV 997	3	2	0	2	3
WV 998	3	2	0	1	3
WV 999	3	2	0	2	3

* 0 = none reported, 1=first quantile, 2=second quantile, 3=up gradient mining
none reported = no report filed for the county or report has no entry

Table 3. Components of the Stream Plethodontid Assemblage Response Index used to calculate the Index of Biotic Integrity (IBI) Score and the results of the performance evaluation of the individual indices (Rocco et al. 2004).

		IBI COMBINATION		
METRIC	METRIC DESCRIPTION	Group 1	Group 2	Group 3
# species	number of Plethodontid species, including woodland species	X	X	
# two-lined	number of *Eurycea bislineata* or *E. cirrigera*	X		
# northern spring	number of *Gyrinophilus porphyriticus*			X
# salamanders	number of salamanders of all species		X	X
# intolerants	number of salamander minus number of *Eurycea spp*.		X	X
# nutrient tolerant	number of *E. spp*. plus *D. fuscus*	X		
# terrestrial	number of salamanders without gills or gill stubs		X	
% EFFICIENCY	All Sites	81	68	71
	Degraded Sites	83	67	74
	Intermediate Sites	81	78	74

Table 4. Literature sources and corresponding parameters for toxicity reference values (TRVs) based on tissue concentrations in aquatic biota used to assess risk to stream salamanders.

Source	Species	Analyte	Wet Weight Concentration (mg/kg)	Effect	Toxicity Measure	Lifestage	HQ TRV
Gillilland et al. 2001	*Rana clamitans*	Aluminum	11.4	Deformities	NOED	Adult	114.0
McGeachy and Dixon 1990	*Oncorhynchus mykiss*	Arsenic	1.5	Mortality	LOED	Juvenile	1.5
Gillilland et al. 2001	*Rana clamitans*	Barium	6	Deformities	NOED	Juvenile	60.0
Nebeker et al. 1995	*Ambystoma gracile*	Cadmium	4.7	Growth	LOED	Larval	4.7
Van De Putte et al. 1981	*Oncorhynchus mykiss*	Chromium VI	8.7	Mortality	LD75	Immature	6.9
Van De Putte et al. 1981	*Oncorhynchus mykiss*	Chromium VI	5.5	Mortality	NOED	Immature	
Gillilland et al. 2001	*Rana clamitans*	Copper	0.28	Deformities	NOED	Juvenile	2.8
Gillilland et al. 2001	*Rana clamitans*	Iron	220	Deformities	NOED	Adult	2200.0
Gillilland et al. 2001	*Rana clamitans*	Lead	0.78	Deformities	NOED	Juvenile	7.8
Fjeld et al. 1998	*Thymallus thymallus*	Mercury	0.27	Behavior	LOED	Adult	0.3
Gillilland et al. 2001	*Rana clamitans*	Nickel	0.122	Deformities	NOED	Adult	1.2
Lemly 2002	*Oncorhynchus mykiss*	Selenium	4	Reproduction	LOED	Adult	4.0
Gillilland et al. 2001	*Rana clamitans*	Zinc	4.6	Deformities	NOED	Adult	46.0

HQ TRV = geometric mean of NOED and LD75 values

selenium (mg/kg dry weight)

Table 5. Life history parameters and toxicity reference values used to calculate risk to mammalian and avian receptors.

Northern Water Shrew						
weight (kg)[a]		food ingestion rate (kg/day)[b]	NOAEL-Hg (mg/kg-day)[c]	LOAEL-Hg (mg/kg-day)[c]	NOAEL-Se (mg/kg-day)[d]	LOAEL-Se (mg/kg-day)[d]
0.0103	0.0163	0.0103	0.15	0.25	0.2	0.33

Louisiana Waterthrush					
weight (kg)[e]	food ingestion rate (kg/day)[f]	NOAEL-Hg (mg/kg-day)[g]	LOAEL-Hg (mg/kg-day)[g]	NOAEL-Se (mg/kg-day)[h]	LOAEL-Se (mg/kg-day)[h]
0.0215	0.0054	0.0064	0.064	0.279	0.336

a - Weight range is an average from three sources - Burt and Grossenheider 1980 (9-14g), PA Game Commission (9.9-17g), and the VA Department of Game and Inland Fisheries (12-18g).
b - Food ingestion rate is equivalent to the body weight as noted by the BC Ministry of Water, Land and Air Protection.
c - Values based on methyl mercury chloride in mink from Wobeser et al.1976 with an endpoint of mortality, weight loss and ataxia, mink selected over rat since both shrews and minks are carnivores. (Oak Ridge)
d - Values based on potassium selenate in rat from Rosenfeld and Beath (1954) with a reproduction endpoint (Oak Ridge)
e - Average bird weight from Environment Canada and the Smithsonian National Zoological Park
f - Rate based on ingestion rate formula for passerine birds, US EPA Wildlife Exposure Factors Handbook, 1993
g - Values based on methyl mercury dicyandiamide in mallard from Heinz (1979) with a reproduction endpoint (Oak Ridge)
h - Values from Handbook of Ecotoxicology, page 486, mallard egg hatchability as a function of selenium concentration in diet, converted to dose using average female mallard weight from US EPA Wildlife Exposure Factors Handbook and allometric equation

Table 6. Summary of salamander collections in Pennsylvania, Virginia and West Virginia, 2003 - 2004.

Species	Common Name	Aquatic Larval Period	Food Habits	Study Areas	Specimens	Samples
Desmognathus fuscus	Northern Dusky	9 months	aquatic invertebrates	43	481	108
Desmognathus monticola	Appalachian Seal	9 months	aquatic invertebrates & salamanders	12	76	34
Desmognathus ochrophaeus	Mountain Dusky	6 months	aquatic invertebrates	6	91	12
Eurycea bislineata	Northern Two-lined	2 years	aquatic invertebrates & eggs	12	113	19
Gyrinophilus porphyriticus	Northern Spring	3 years	salamanders & aquatic invertebrates	6	13	8
Plethodon cinereus	Redback	none	terrestrial invertebrates	1	5	1
				TOTAL	779	182

Table 7. Selenium, mercury, aluminum, and copper concentrations, hazard quotients (HQs), and resulting study area classifications by tissue concentrations.

	mean [Se]	Se HQ		mean [Hg]	Hg HQ		mean [Al]	Al HQ		mean [Cu]	Cu HQ		HQ
SITE ID	mg/kg dw	mean	range	mg/kg ww	mean	range	mg/kg ww	mean	range	mg/kg ww	mean	range	CLASS*
PA 035	2.49	0.6	0.5-0.9	0.049	0.2	0.1-0.4	138.3	1.2	0.8-1.9	1.78	0.6	0.3-1.2	2
PA 082	2.20	0.6	0.1-0.8	0.025	0.1	0.1-0.1	120.4	1.1	0.5-1.6	1.06	0.4	0.3-0.4	2
PA 410	4.13	1.0	1.0	0.026	0.1	0.1	112.7	1.0	1.0	0.98	0.4	0.4	2
PA 411	2.41	0.6	0.5-0.8	0.029	0.1	0.1-0.1	36.7	0.3	0.1-0.5	0.74	0.3	0.2-0.3	1
PA 412	5.24	1.3	0.9-2.7	0.026	0.1	0.1-0.2	73.6	0.6	0.1-1.0	0.90	0.3	0.2-0.5	2
PA 413	3.94	1.0	1.0-1.0	0.041	0.2	0.1-0.2	77.3	0.7	0.7	0.95	0.5	0.5	2
PA 522	2.88	0.7	0.5-1.3	0.052	0.2	0.1-1.1	178.2	1.6	0.3-4.2	1.41	0.5	0.3-1.0	2
PA 544	2.10	0.5	0.1-1.1	0.110	0.4	0.1-1.1	149.4	1.3	1.0-1.6	1.56	0.6	0.3-0.9	2
PA 771	2.49	0.6	0.5-0.9	0.025	0.1	0.1-0.1	103.6	0.9	0.7-1.1	0.86	0.3	0.3-0.3	1
PA 787	2.10	0.5	0.1-0.8	0.040	0.1	0.1-0.4	41.3	0.4	0.3-0.4	2.02	0.7	0.3-1.4	1
PA 848	2.19	0.5	0.5-0.6	0.031	0.1	0.1-0.1	27.7	0.2	0.1-0.5	0.87	0.3	0.2-0.4	1
VA 508	3.16	0.8	0.6-1.2	0.023	0.1	0.1-0.1	24.0	0.2	0.1-0.3	0.78	0.3	0.3-0.3	1
VA 554	1.57	0.4	0.1-0.9	0.025	0.1	0.1-0.10	69.6	0.6	0.4-0.9	0.84	0.3	0.3-0.3	1
VA 990	1.16	0.3	0.3-0.3	0.020	0.1	0.1-0.1	26.4	0.2	0.1-0.4	1.00	0.4	.03-0.5	1
VA 991	1.35	0.3	0.3-0.4	0.036	0.1	0.1-0.2	33.6	0.3	0.1-0.5	1.37	0.5	0.3-0.7	1
VA 992	1.94	0.5	0.3-0.7	0.019	0.1	0.0-0.1	57.0	0.5	0.2-1.2	1.19	0.4	0.2-0.8	1
VA 993	4.37	1.1	0.5-1.8	0.034	0.1	0.1-0.2	211.6	1.9	1.1-3.3	5.41	1.9	0.3-5.6	2
VA 994	3.06	0.8	0.6-1.4	0.024	0.1	0.1-0.1	69.0	0.6	0.4-.08	0.47	0.2	0.1-0.2	1
VA 995	3.02	0.8	0.6-1.0	0.027	0.1	0.1-0.1	52.6	0.5	0.4-0.5	1.16	0.4	0.3-0.5	1
VA 996	2.55	0.6	0.5-0.8	0.021	0.1	0.1-0.1	32.0	0.3	0.3	0.80	0.3	0.3	1
VA 997	2.55	0.6	0.5-0.8	0.023	0.1	0.1-0.1	114.8	1.0	1.0	0.77	0.3	0.3	2
VA 998	1.89	0.5	0.1-1.3	0.050	0.2	0.1-0.6	68.9	0.6	0.3-0.9	1.63	0.6	0.3-1.0	1
VA 999	1.77	0.4	0.1-1.0	0.044	0.2	0.1-0.5	281.3	2.5	0.5-4.8	1.39	0.5	0.3-0.8	2
WV 018	10.37	2.6	1.4-3.6	0.049	0.2	0.1-0.5	191.8	1.7	0.4-2.9	6.79	2.4	0.3-5.0	2
WV 025	5.40	1.3	0.6-2.0	0.031	0.1	0.1-0.2	143.6	1.3	0.8-1.7	1.62	0.6	0.2-0.9	2
WV 039	2.88	0.7	0.5-1.0	0.027	0.1	0.1-0.1	39.5	0.3	0.2-0.4	1.14	0.4	0.3-0.5	1
WV 085	6.56	1.6	1.4-2.2	0.051	0.2	0.1-0.3	261.5	2.3	1.5-2.7	4.24	1.5	0.4-2.5	2
WV 088	7.79	1.9	1.9	0.027	0.1	0.1	na	na	na	na	na	na	2
WV 090	4.11	1.0	0.7-1.4	0.025	0.1	0.1-0.10	21.3	0.2	0.2	0.47	0.2	0.2	2

Table 7. Selenium, mercury, aluminum, and copper concentrations, hazard quotients (HQs), and resulting study area classifications by tissue concentrations (cont.).

	mean [Se]	Se HQ		mean [Hg]	Hg HQ		mean [Al]	Al HQ		mean [Cu]	Cu HQ		HQ
SITE ID	mg/kg dw	mean	range	mg/kg ww	mean	range	mg/kg ww	mean	range	mg/kg ww	mean	range	CLASS*
WV 092	2.23	0.6	0.6	0.031	0.1	0.113	na	na	na	na	na	na	1
WV 093	2.50	0.6	0.6-0.7	0.019	0.1	0.1-0.1	na	na	na	na	na	na	1
WV 504	2.97	0.7	0.5-1.1	0.025	0.1	0.1-0.1	36.9	0.3	0.3-0.4	0.60	0.2	0.2-0.2	1
WV 505	1.27	0.3	0.3-0.3	0.036	0.1	0.1-0.1	74.5	0.7	0.2-1.2	0.68	0.2	0.2-0.3	1
WV 982	2.30	0.6	0.5-0.7	0.016	0.1	0.1-0.1	122.5	1.1	0.9-1.2	0.59	0.2	0.2-0.2	2
WV 983	2.05	0.5	0.5	0.013	0.0	0.1	40.8	0.4	0.4	0.66	0.2	0.2	1
WV 984	2.05	0.5	0.5-0.5	0.032	0.1	0.1-0.1	27.6	0.2	0.2-0.3	0.94	0.3	0.3-0.4	1
WV 985	2.23	0.6	0.6-0.6	0.025	0.1	0.1-0.1	45.5	0.4	0.4-0.4	0.57	0.2	0.2-0.2	1
WV 986	6.04	1.5	1.2-2.0	0.016	0.1	0.1-0.1	22.3	0.2	0.1-0.2	2.96	1.1	0.4-1.5	2
WV 987	3.19	0.8	0.7-0.9	0.038	0.1	0.1-0.2	101.0	0.9	0.5-1.3	3.99	1.4	0.3-2.6	2
WV 989	1.63	0.4	0.4-0.4	0.020	0.1	0.1-0.1	30.1	0.3	0.3-0.3	0.53	0.2	0.2-0.2	1
WV 990	2.31	0.6	0.6	0.017	0.1	0.1	31.5	0.3	0.3	1.29	0.5	0.5	1
WV 991	4.02	1.0	0.7-1.3	0.010	0.0	0.0-0.0	43.6	0.4	0.3-0.5	1.22	0.4	0.3-0.6	2
WV 992	2.00	0.5	0.5-0.6	0.015	0.1	0.1-0.1	60.1	0.5	0.4-0.7	1.27	0.5	0.4-0.5	1
WV 993	5.42	1.4	1.3-1.4	0.012	0.0	0.0-0.1	134.5	1.2	1.1-1.3	1.12	0.4	0.4-0.4	2
WV 994	2.61	0.7	0.4-0.9	0.027	0.1	0.1-0.1	34.3	0.3	0.1-0.8	0.85	0.3	0.2-0.4	1
WV 995	6.91	1.7	1.2-2.4	0.013	0.0	0.0-0.1	71.2	0.6	0.5-0.8	1.17	0.4	0.4-0.4	2
WV 996	3.91	1.0	0.6-1.3	0.029	0.1	0.1-0.1	67.1	0.6	0.6	1.78	0.6	0.6	2
WV 997	6.53	1.6	1.0-2.4	0.022	0.1	0.0-0.1	63.9	0.6	0.3-0.8	11.96	4.3	2.0-6.6	2
WV 998	4.48	1.1	0.7-1.5	0.023	0.1	0.1-0.1	20.8	0.2	0.2	0.94	0.3	0.3	2
WV 999	2.25	0.6	0.6	0.045	0.2	0.2	na	na	na	na	na	na	1

mean hazard quotient greater than 1

individual composites with hazard quotients greater than 1

*1=hazard quotient<1, 2=hazard quotient>1

Table 8. SPAR Index of Biotic Integrity (IBI) scores and status with respect to hazard quotient (HQ) and potential source classes.

SITE ID	HQ CLASS*	SOURCE CLASS**	HABITAT CLASS***	SPAR IBI SCORE****	STATUS
PA 544	2	1	GRP 1	1.67	IMP
PA 787	1	1	GRP 1	6.67	REF
PA 035	2	2	GRP 3	6.67	REF
PA 082	2	2	GRP 1	10.00	REF
PA 410	2	2	GRP 1	10.00	REF
PA 411	1	2	GRP 1	6.67	REF
PA 412	2	2	GRP 1	5.00	INTMED
PA 522	2	2	GRP 3	1.67	IMP
PA 771	1	2	GRP 1	1.67	IMP
PA 848	1	2	GRP 1	10.00	REF
VA 508	1	2	GRP 3	10.00	REF
VA 554	1	2	GRP 3	6.67	REF
VA 990	1	2	GRP 3	10.00	REF
VA 995	1	2	GRP 3	10.00	REF
VA 996	1	2	GRP 3	8.33	REF
VA 997	2	2	GRP 3	8.33	REF
VA 998	1	2	GRP 3	6.67	REF
WV 039	1	2	GRP 3	10.00	REF
WV 092	1	2	GRP 3	8.33	REF
WV 504	1	2	GRP 3	5.00	INTMED
WV 505	1	2	GRP 3	3.33	IMP
WV 983	1	2	GRP 3	6.67	REF
WV 994	1	2	GRP 3	6.67	REF
VA 991	1	3	GRP 3	5.00	INTMED
VA 992	1	3	GRP 3	6.70	REF
VA 993	2	3	GRP 2	1.25	IMP
VA 994	1	3	GRP 3	5.00	INTMED
VA 999	2	3	GRP 3	1.67	IMP
WV 090	2	3	GRP 3	1.67	IMP
WV 997	2	3	GRP 3	0.00	IMP
WV 998	2	3	GRP 3	0.00	IMP
WV 999	1	3	GRP 3	0.00	IMP

*1=hazard quotient<1, 2=hazard quotient>1
** 0 = none reported, 1=first quantile, 2=second quantile, 3=downstream of valley fill
***group class based on latitude, % boulder cover, stream temperature, and % slope
****scoring is group specific – see Table 3

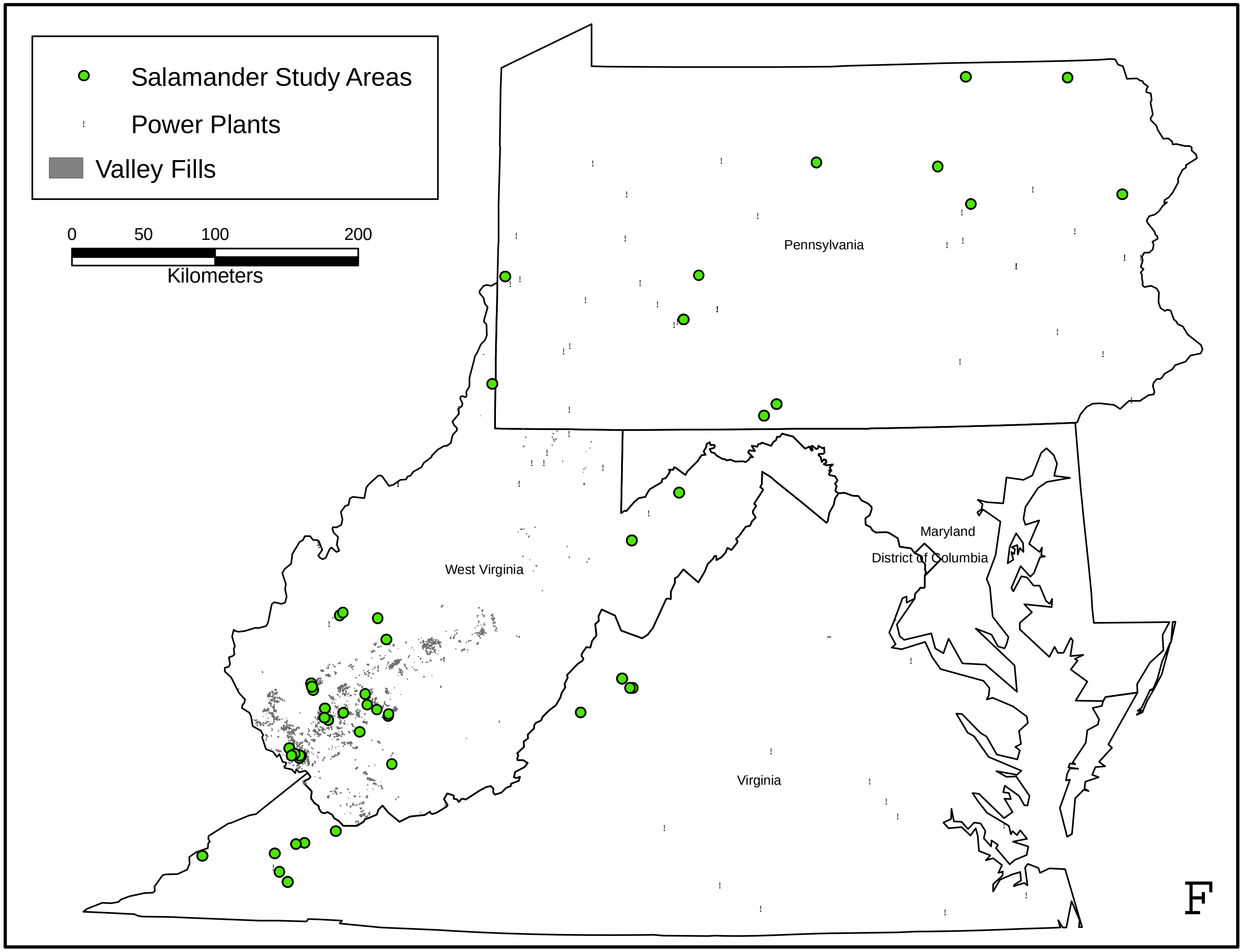


Figure 1. Study areas in Pennsylvania, West Virginia, and Virginia relative to power plants and valley fills. Note – valley fills are not depicted for Virginia sites and power plants are not depicted for Maryland.

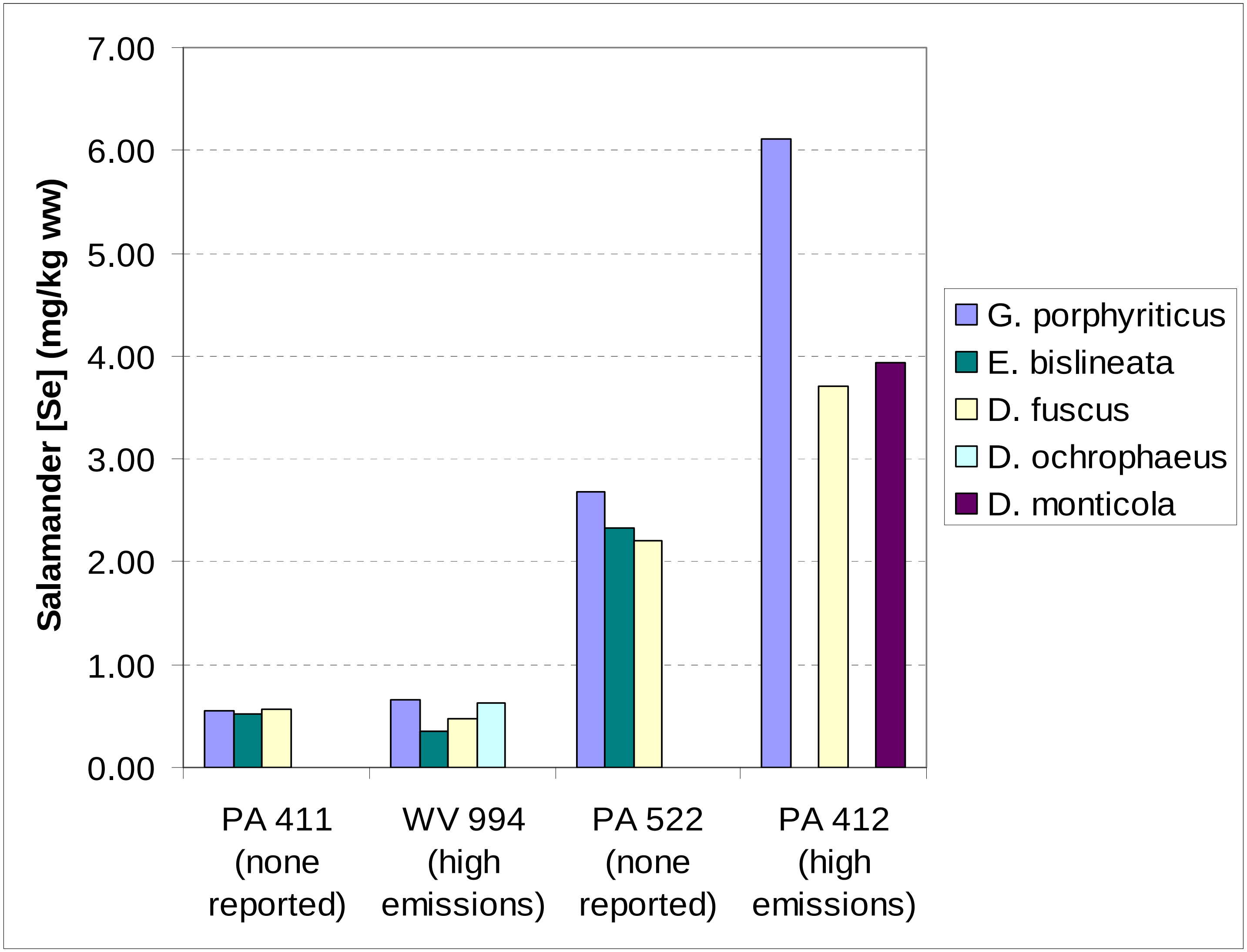


Figure 2. Comparison of average salamander selenium concentrations across species at study areas (selenium source class) which had *G. porphyriticus* and a minimum of two additional species.

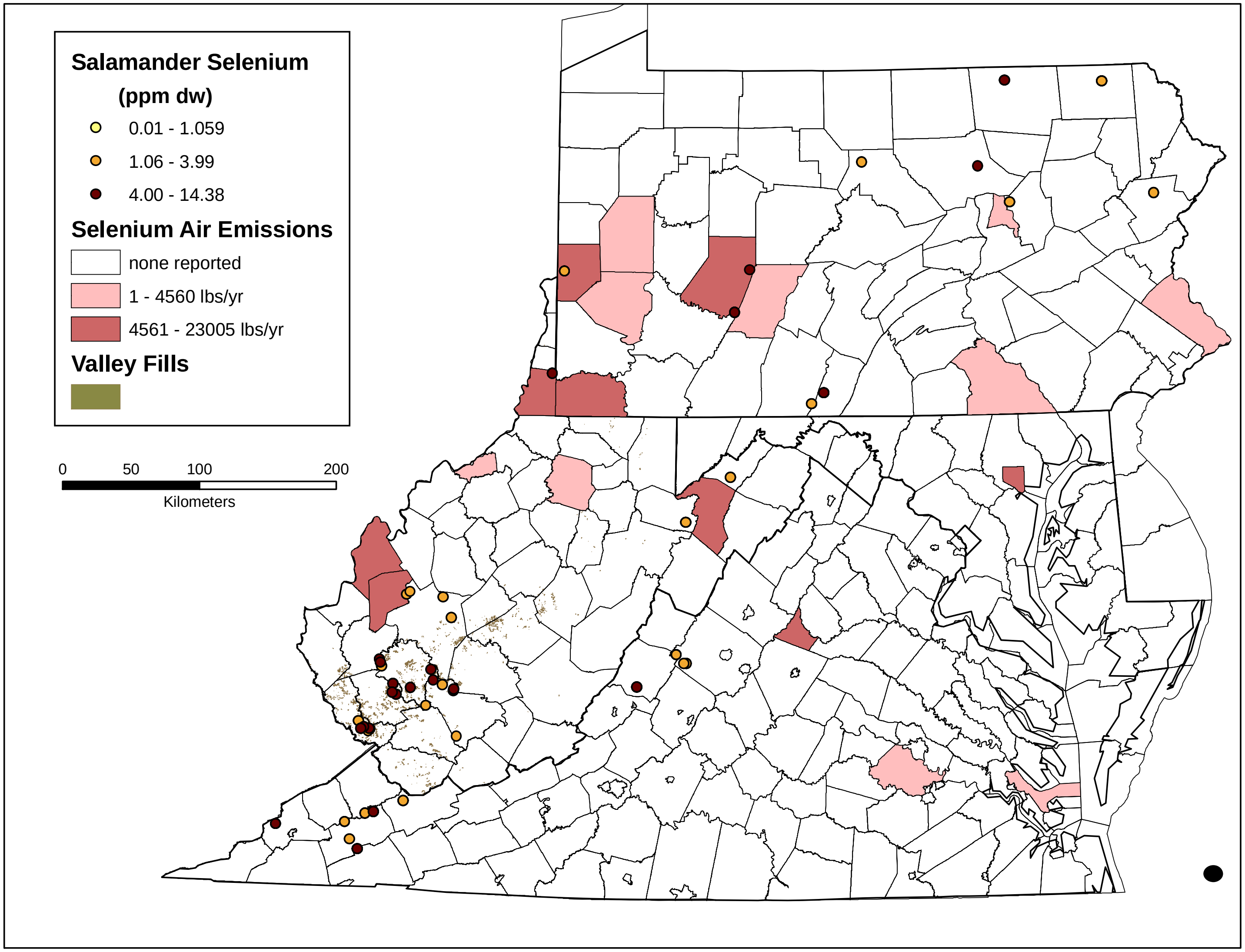


Figure 3. Concentrations of selenium in whole body samples of salamanders relative to selenium air emissions (2003 US EPA Toxic Release Inventory). Low and medium Se categories = HQ<1; high Se category = HQ>1.

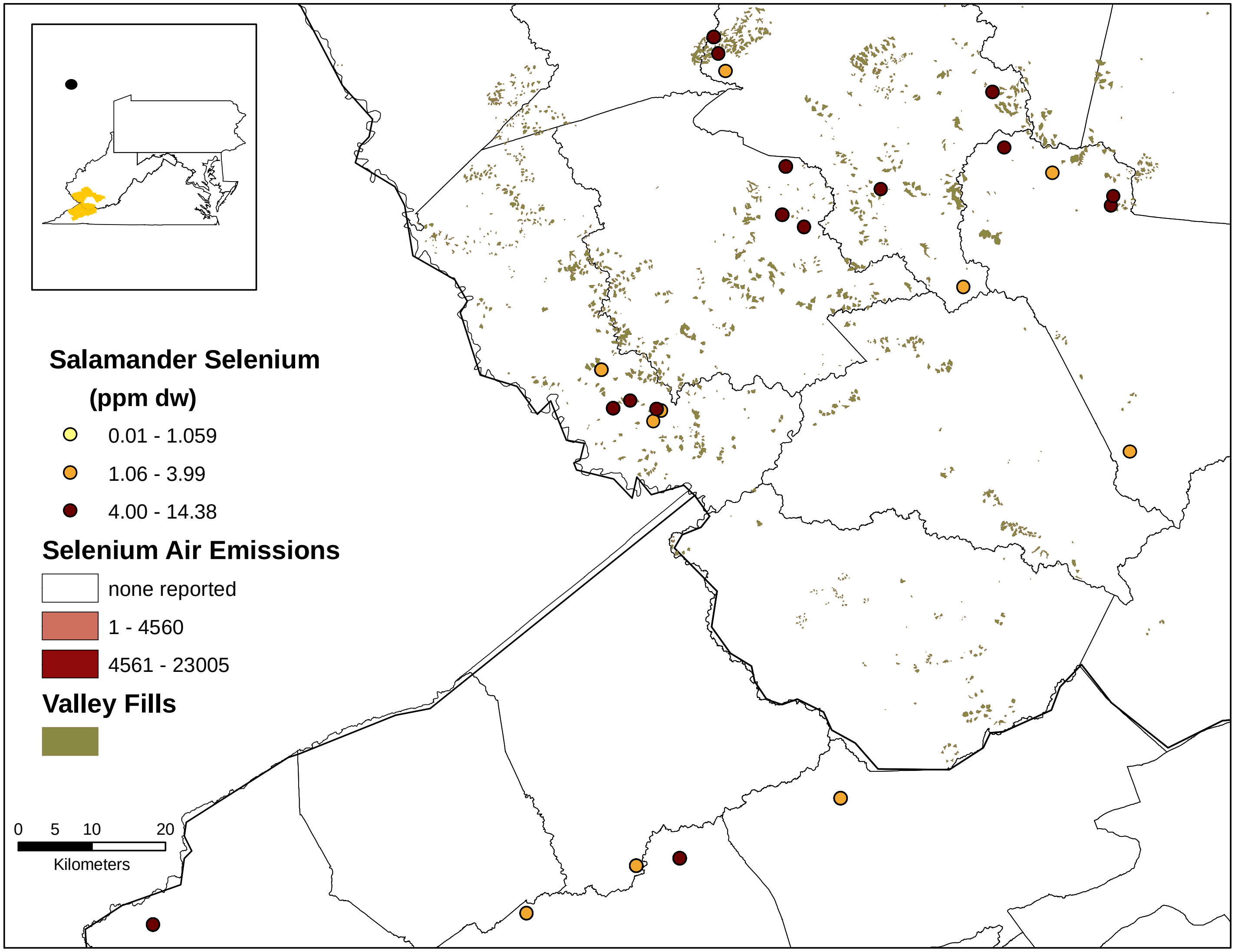


Figure 4. Expanded area of southwestern Virginia and West Virginia depicting concentrations of selenium in whole body samples of salamanders.

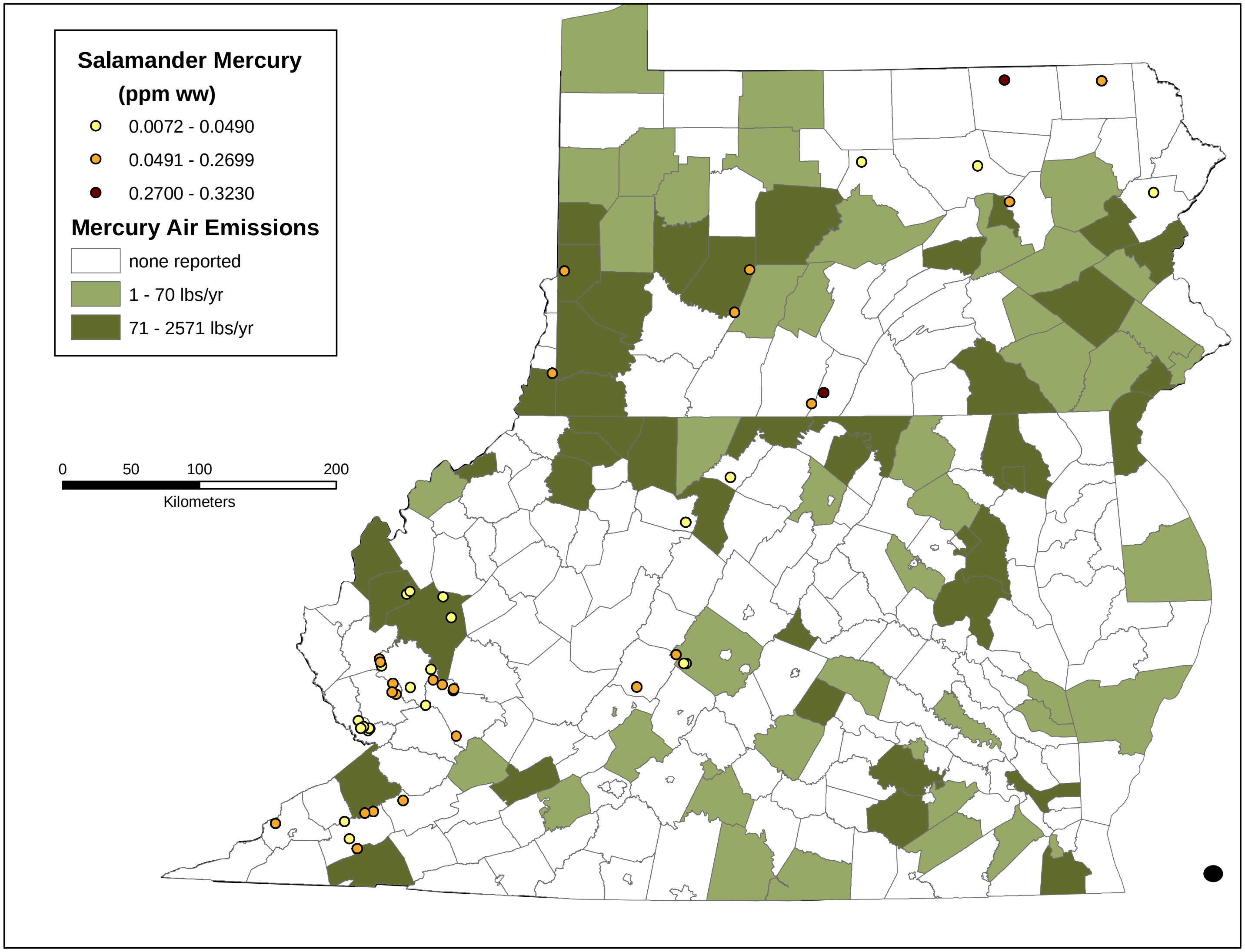


Figure 5. Concentrations of mercury in whole body samples of salamanders relative to mercury air emissions (2003 US EPA Toxic Release Inventory). Low and medium Hg categories = HQ<1; high Hg category = HQ>1.

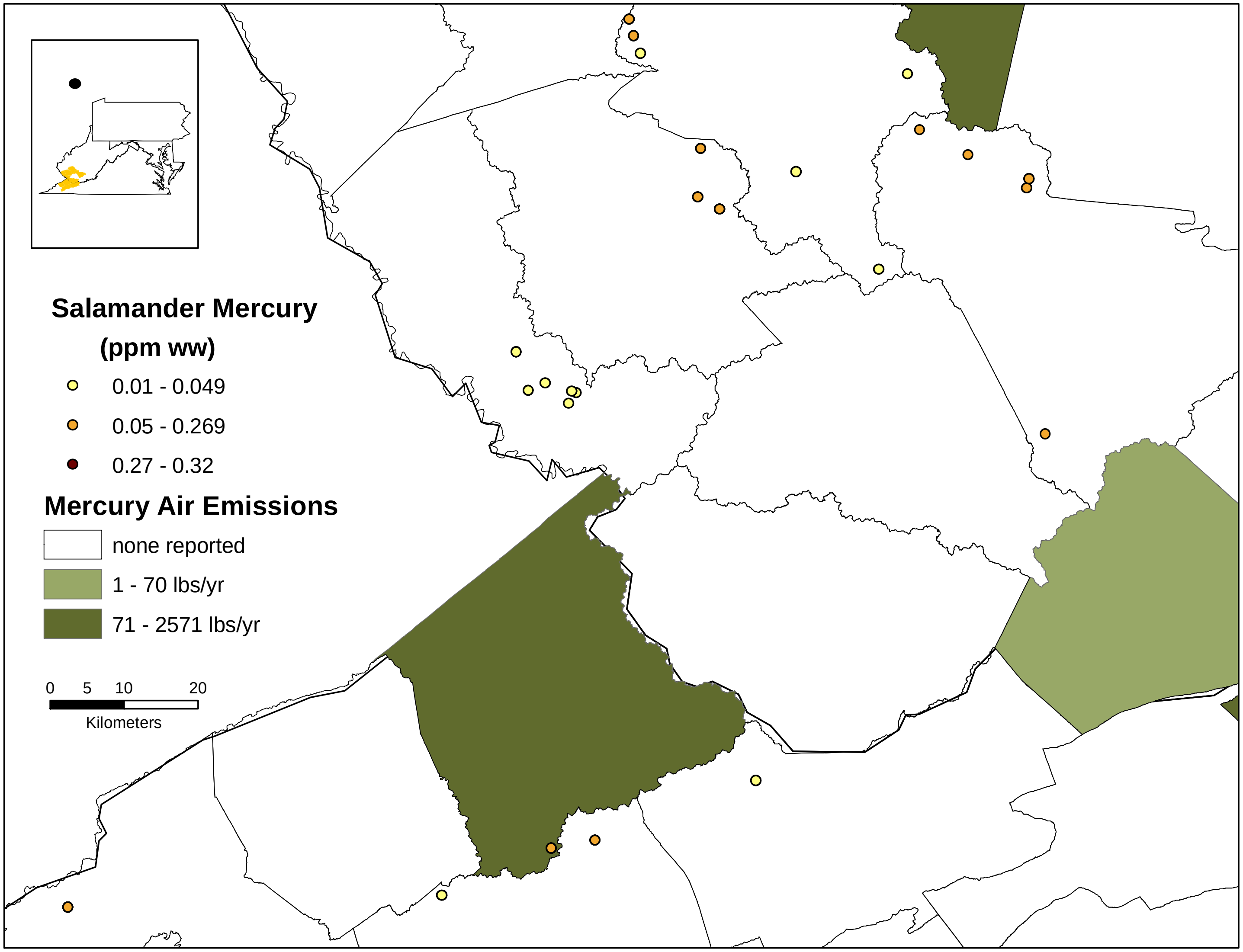


Figure 6. Expanded area of southwestern Virginia and West Virginia depicting concentrations of mercury in whole body samples of salamanders.

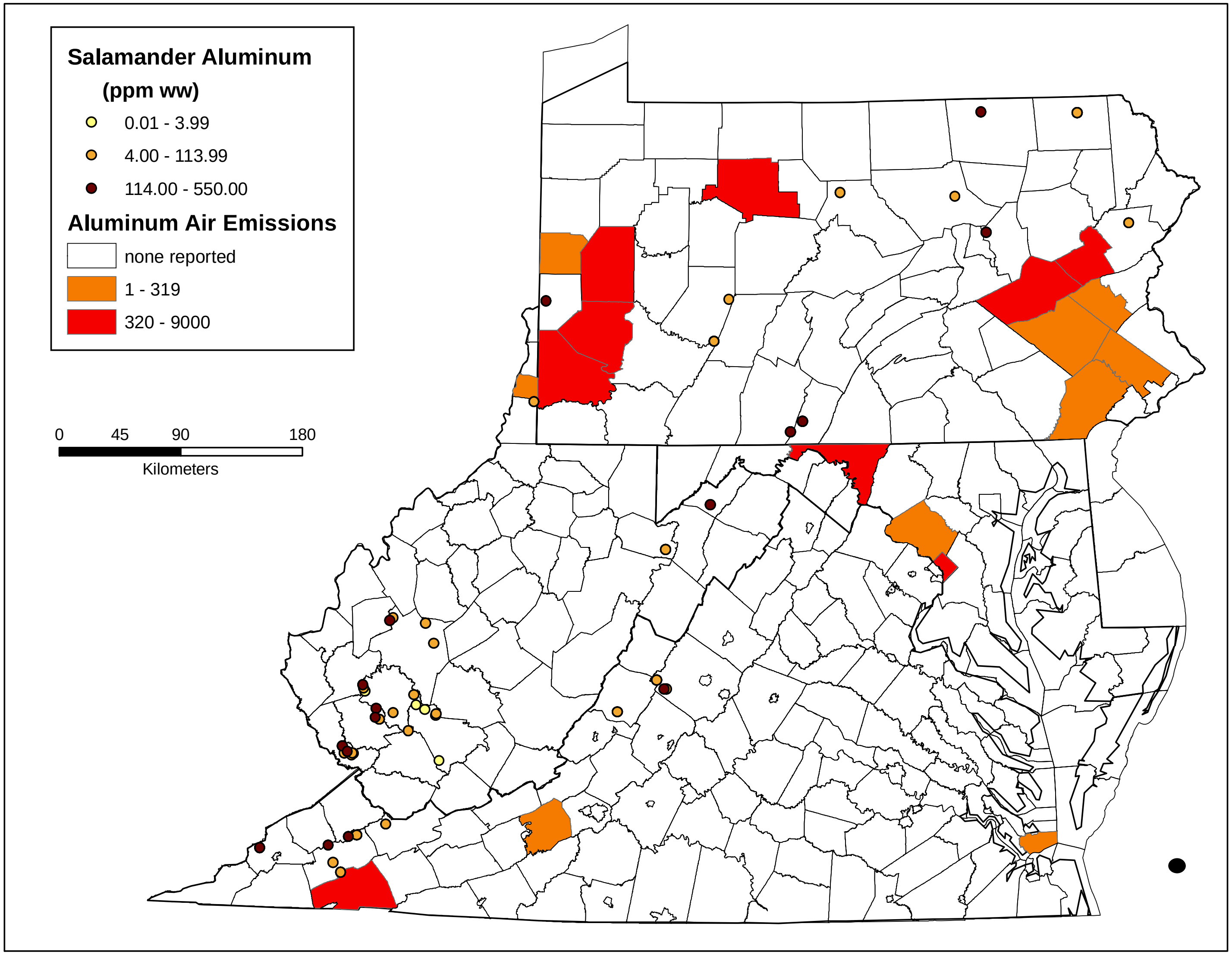


Figure 7. Concentrations of aluminum in whole body samples of salamanders relative to aluminum air emissions (2003 US EPA Toxic Release Inventory).

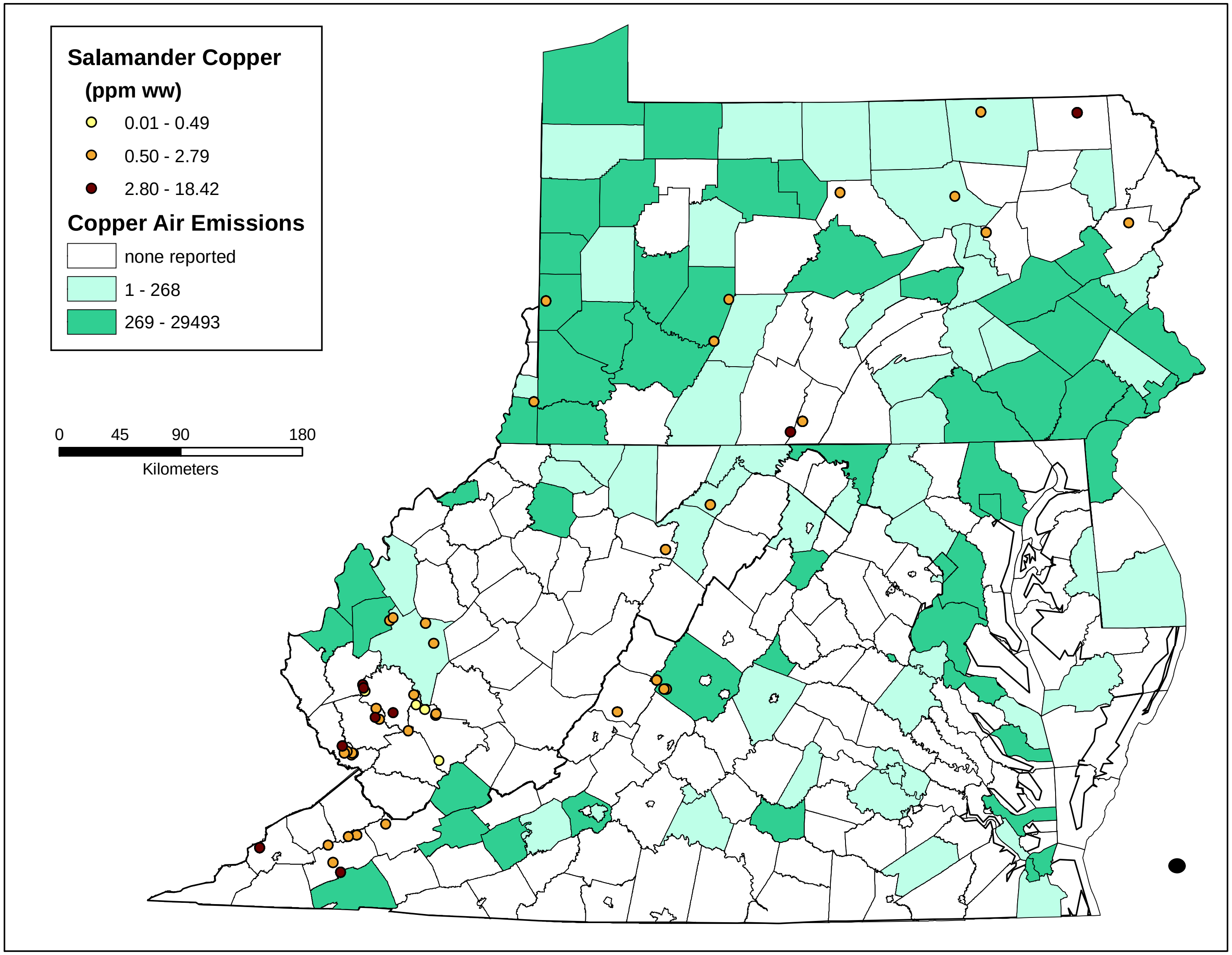


Figure 8. Concentrations of copper in whole body samples of salamanders relative to copper air emissions (2003 US EPA Toxic Release Inventory).

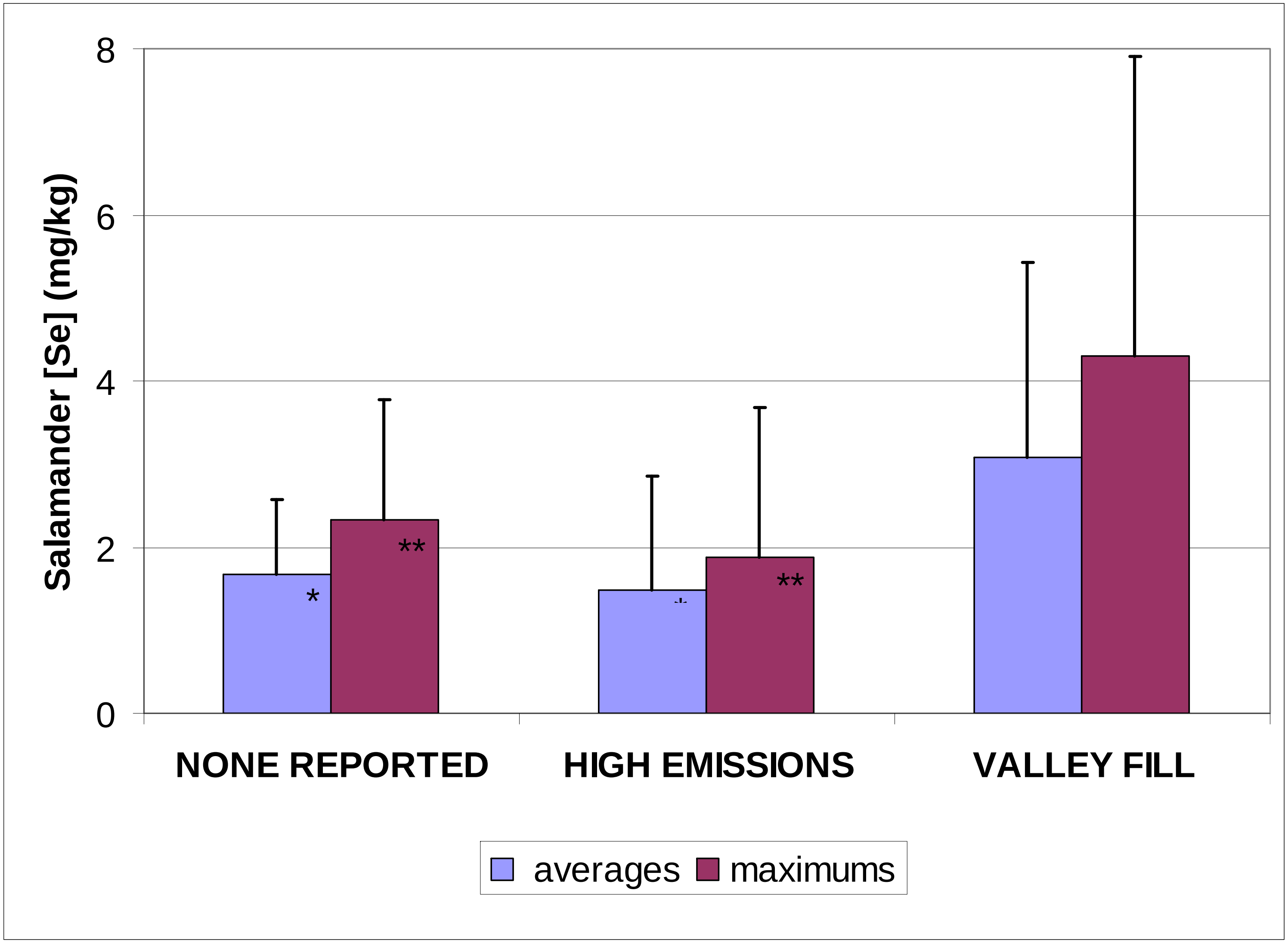


Figure 9. Mean and standard deviation for average and maximum salamander selenium concentrations based on selenium sources. * and ** None reported and high emissions study areas differ significantly from respective valley fill study areas at $p<0.05$ based on ANOVA and Dunnett's.

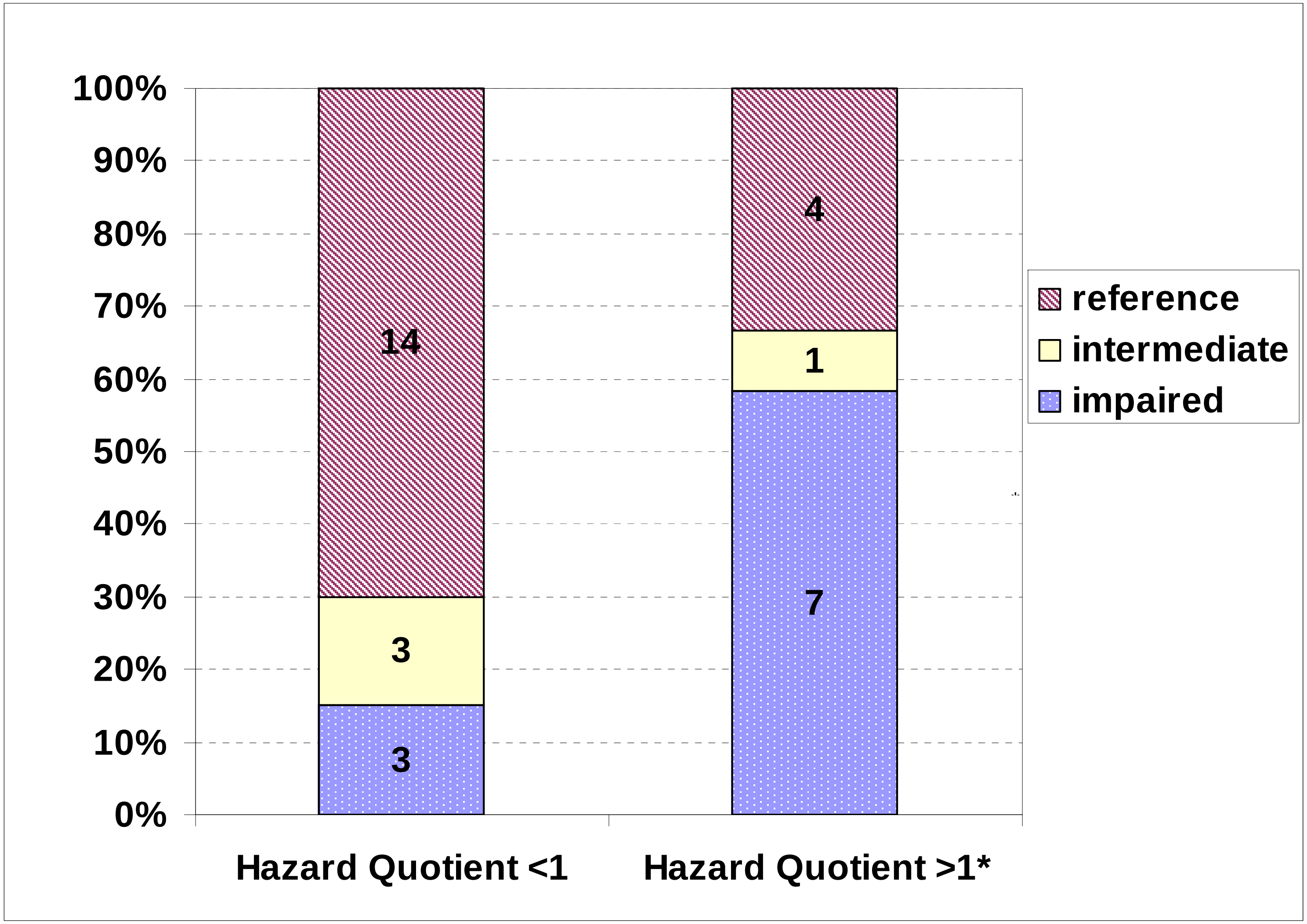


Figure 10. Proportion of salamander assemblages in each IBI category relative to combined inorganic contaminant HQs. Bold numbers represent number of salamander assemblages. * Study areas assemblages with HQs>1 differ significantly ($p=0.038$) from assemblages at HQ<1 study areas based on Chi-square analysis.

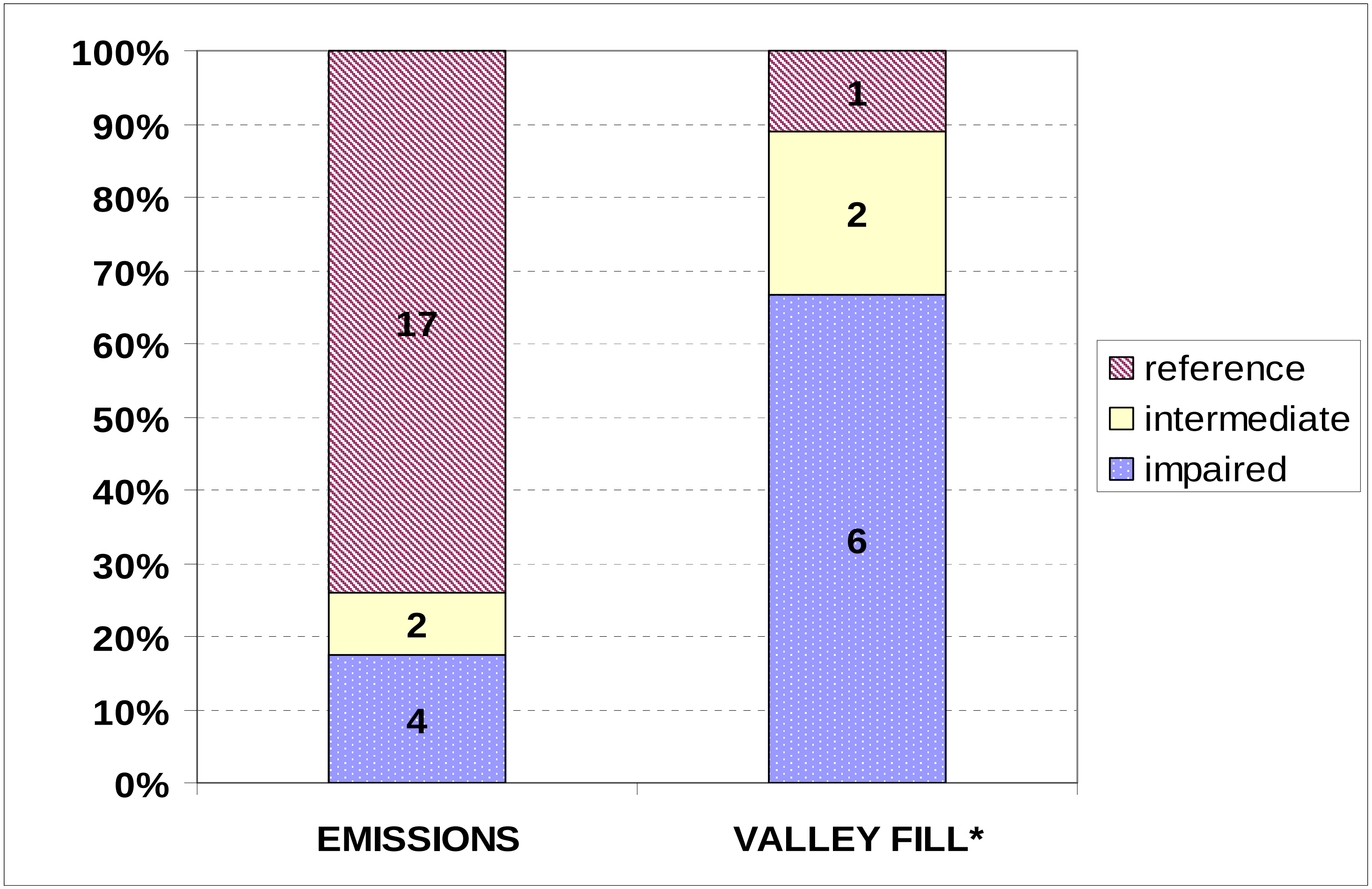


Figure 11. Proportion of salamander assemblages in each IBI category relative to potential contaminant sources. Bold numbers represent number of salamander assemblages. * Valley fill assemblages differ significantly ($p=0.005$) from emission source assemblages based on Chi-square analysis.

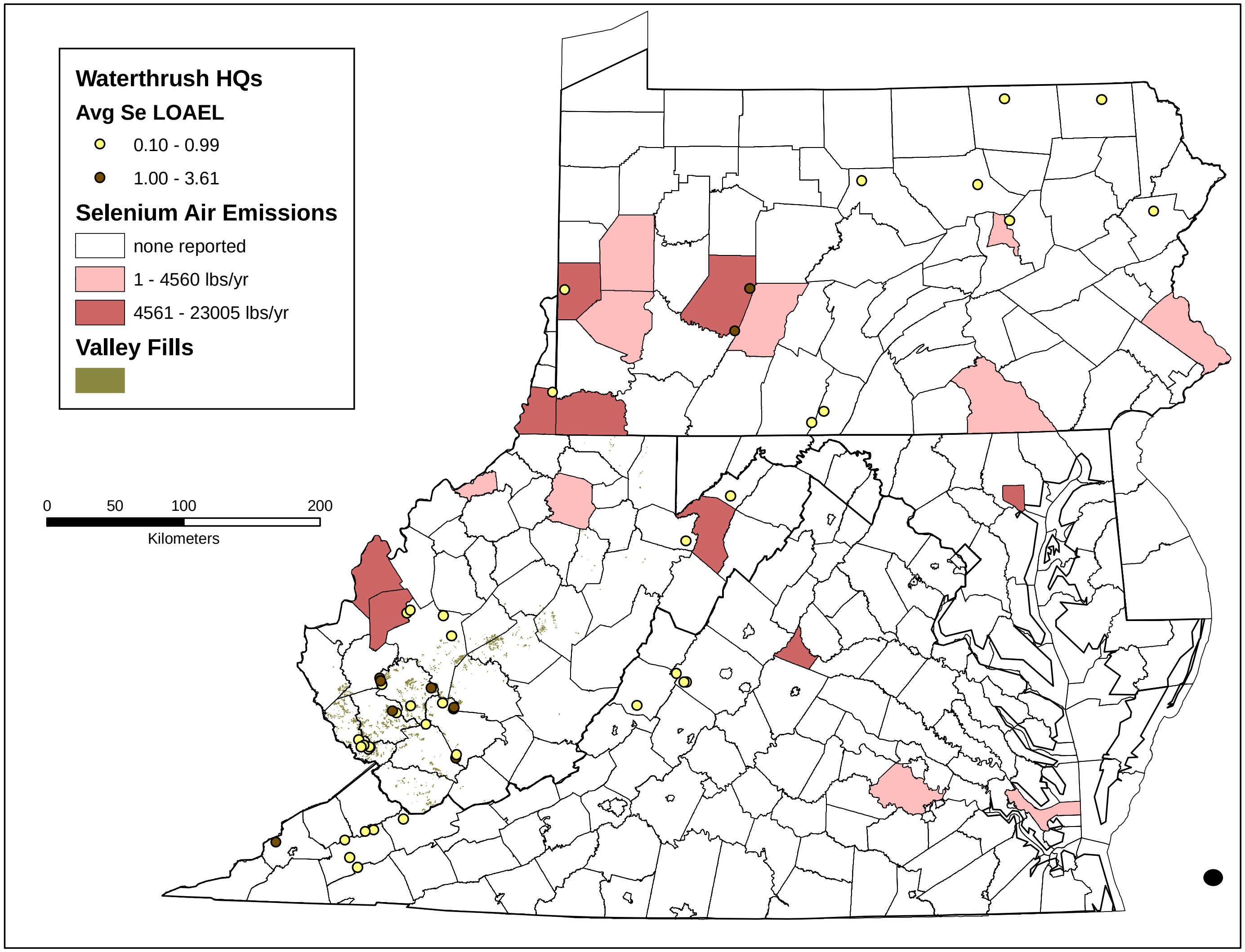


Figure 12. Waterthrush selenium hazard quotients (HQs) based on average salamander concentrations and lowest observable effect toxicity values relative to potential selenium sources. Note – valley fills are not depicted for Virginia sites.

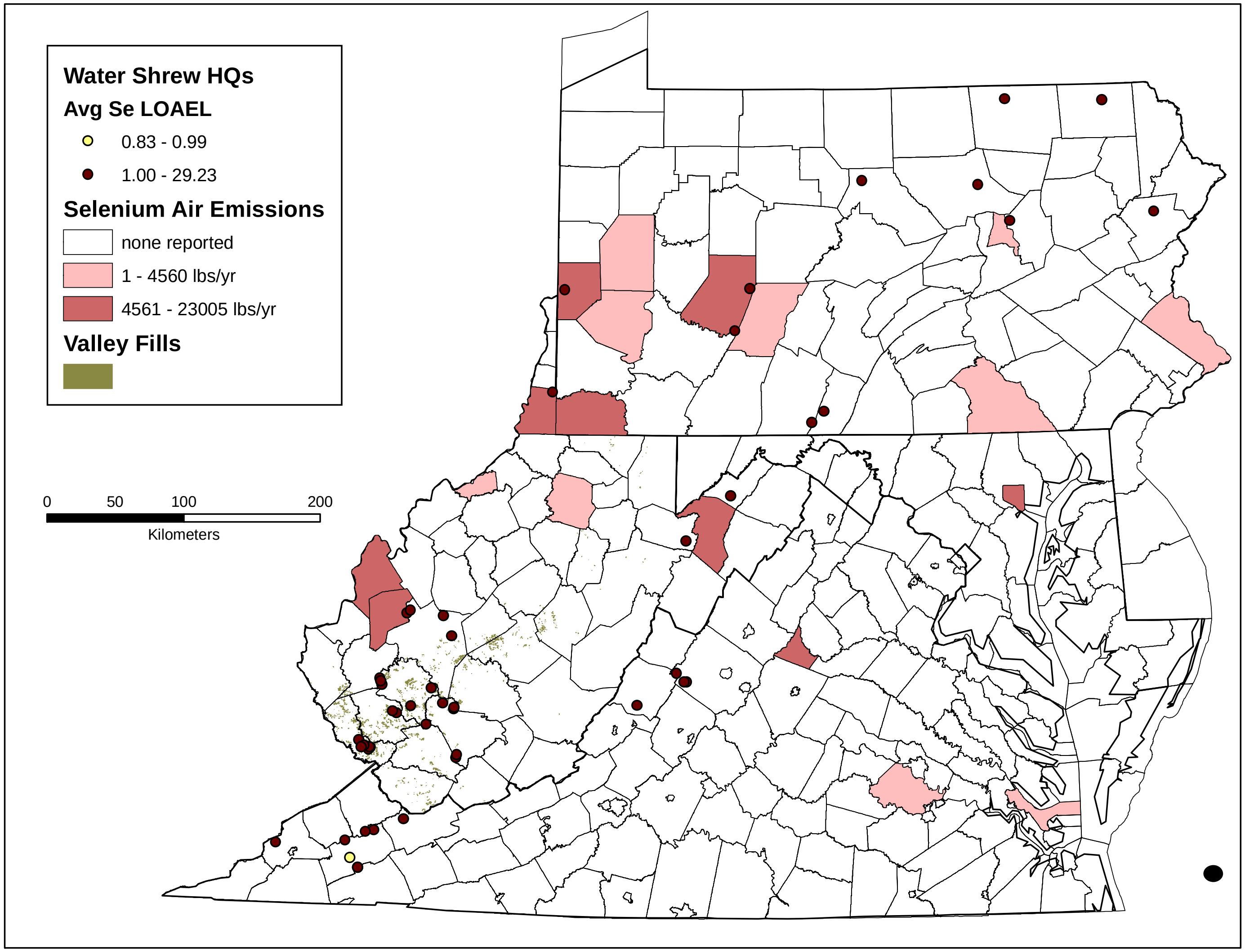


Figure 13. Water shrew selenium hazard quotients (HQs) based on average salamander concentrations and lowest observable effect level relative to potential selenium sources. Note – valley fills are not depicted for Virginia sites and water shrew not documented in southwest West Virginia counties.

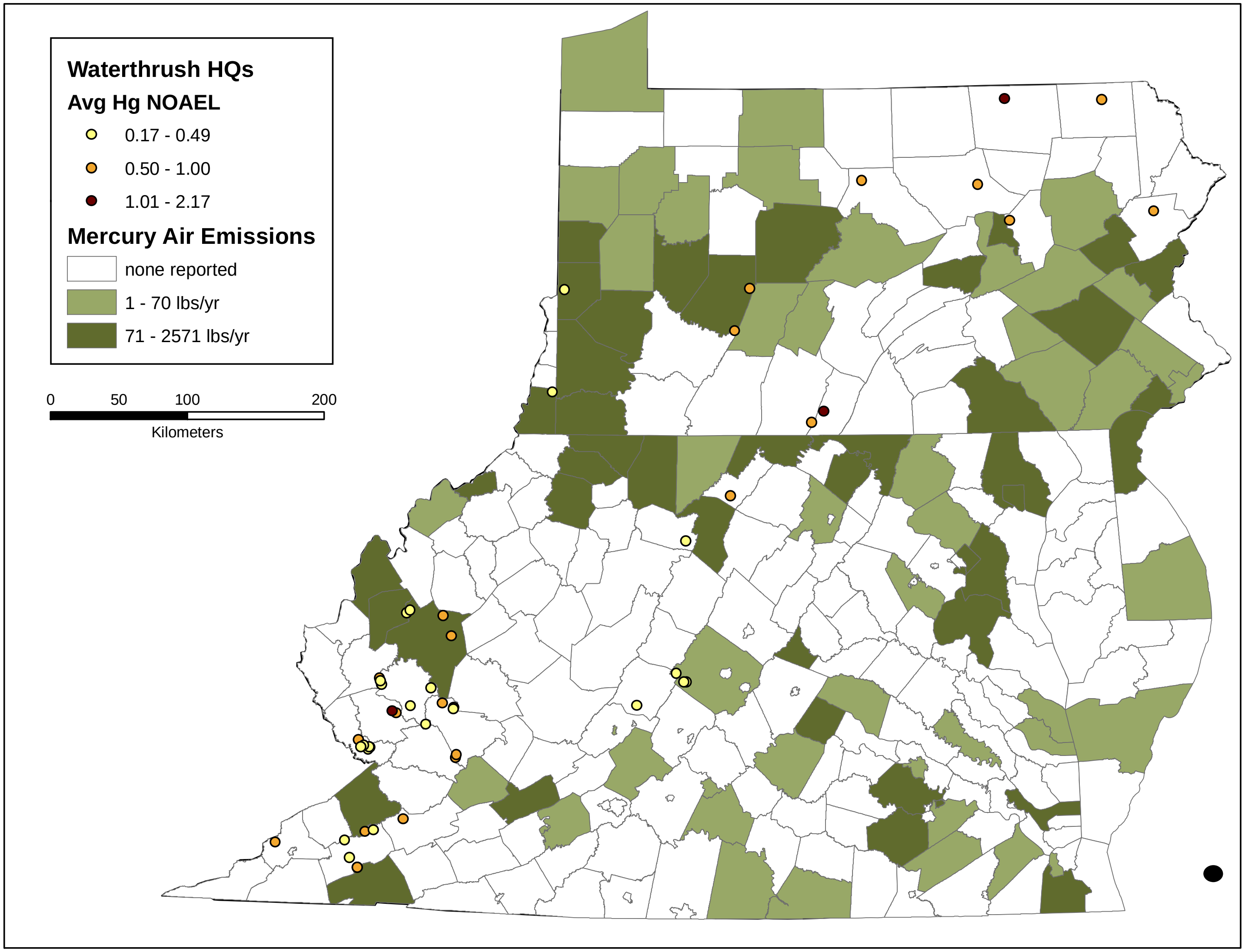


Figure 14. Waterthrush mercury hazard quotients (HQs) based on average salamander concentrations and highest no observable effect toxicity values relative to potential mercury sources.

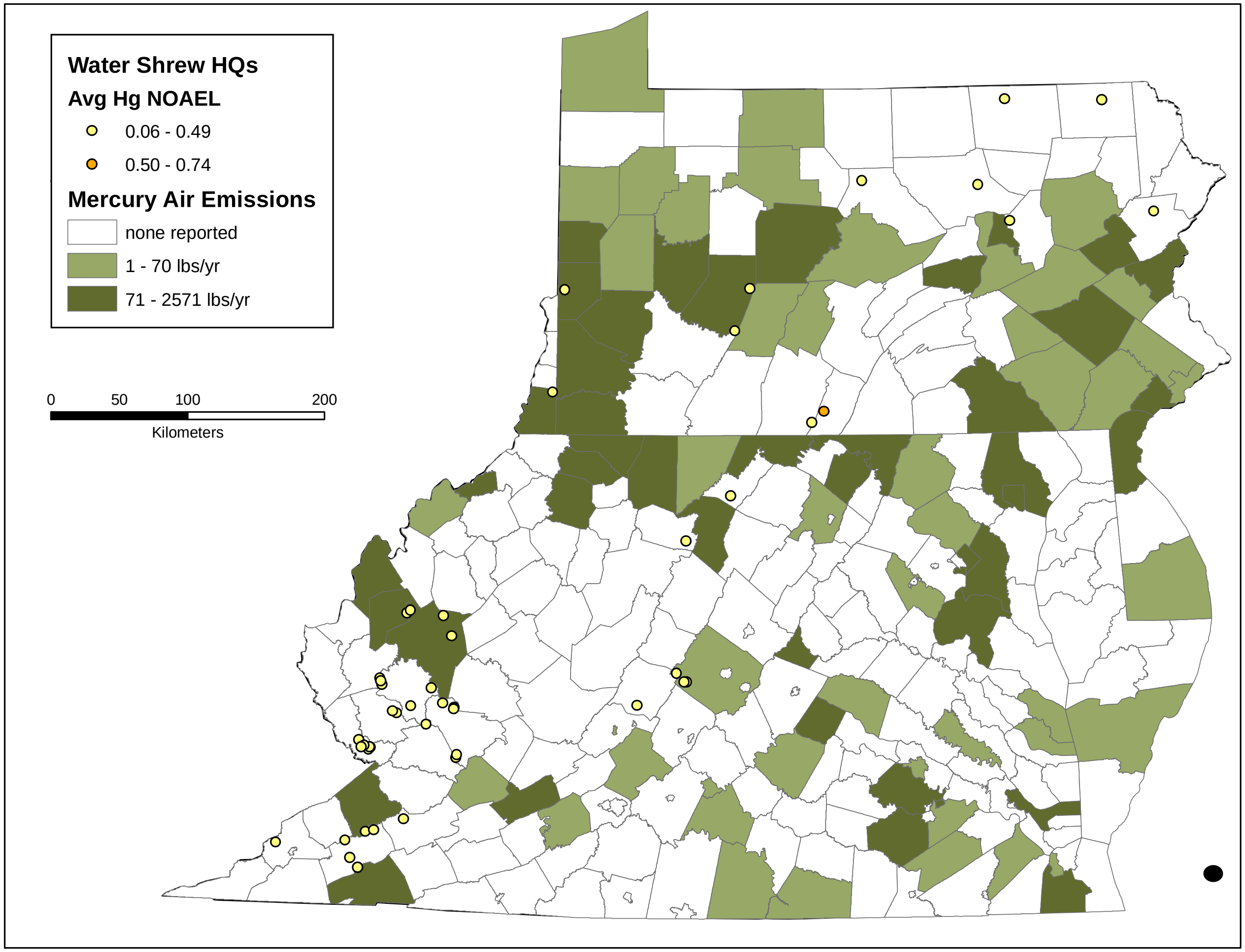
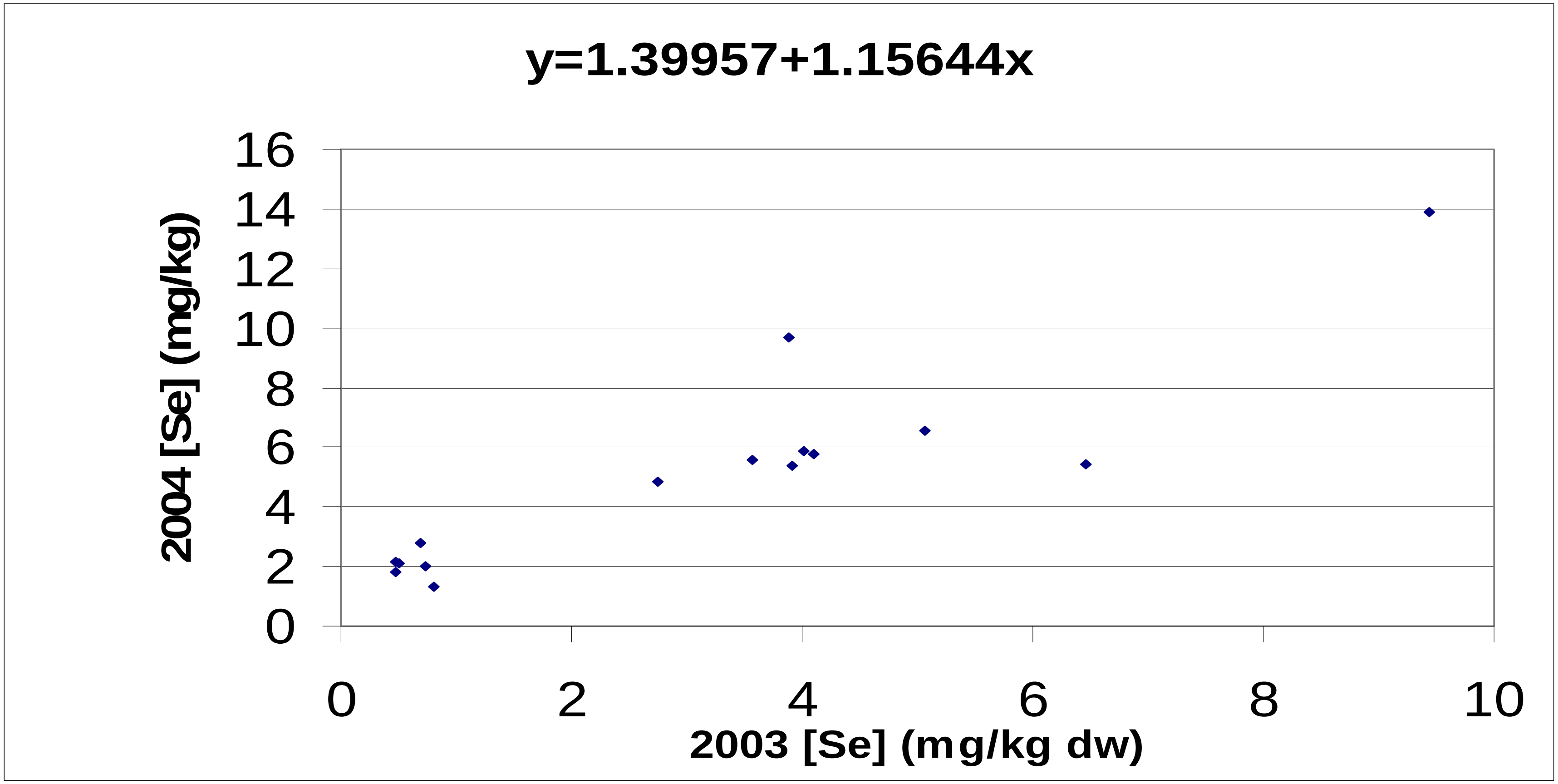


Figure 15. Water shrew mercury hazard quotients (HQs) based on average salamander concentrations and highest no observable effect toxicity values relative to potential mercury sources. Note – water shrew not documented in southwest West Virginia counties.

APPENDICES

Appendix A: Relationship between duplicate samples from laboratories used in 2003 and 2004. Regression equation used to correct for differences between the 2004 (x) and the 2003 (y) samples.



Appendix A. Linear Regression Summary Output

Regression Statistics	
Multiple R	0.897101368
R Square	0.804790864
Adjusted R Square	0.789774777
Standard Error	1.547922028
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	128.4175595	128.4176	53.59524	5.82427E-06
Residual	13	31.14881388	2.396063		
Total	14	159.5663733			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.399571338	0.635351962	2.202828	0.046257	0.026977138	2.77216554	0.026977138	2.772165538
X Variable 1	1.156438735	0.157964503	7.320877	5.82E-06	0.815177241	1.49770023	0.815177241	1.49770023

Appendix B: Analytes, tissue composite concentrations, and detection limits.

Available electronically upon request from:

Kathleen Patnode
U.S. Fish and Wildlife Service
c/o U.S. EPA
1060 Chapline St Suite 303
Wheeling WV 26003-2995
kathleen_patnode@fws.gov