A contribution of the IUCN/SSC Conservation Breeding Specialist Group

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EXECUTIVE SUMMARY

Salamanders, along with many other amphibian species have been declining in recent years. The IUCN lists 47% of the world’s salamanders threatened or endangered, yet few people know that the Appalachian region of the United States is home to 14% of the world’s 535 salamander species, making it an extraordinary salamander biodiversity hotspot, and a priority region for salamander conservation. Observations within the Appalachians suggest that salamanders are declining. Populations of 38 species of *Plethodon* salamanders declined by 50% in the 1990s, and the causes remain unknown.

A workshop hosted by the Smithsonian’s National Zoological Park was convened 30 and 31 May 2008, and 35 salamander experts were asked to identify prime threats to salamanders and conservation actions that could be taken to protect this unique feature of America’s biodiversity. The experts pointed to climate change, pollution, residential development, energy production and mining, and invasive species and disease as the top threats to salamanders in the Appalachians. While they suspected that these threats might severely impact salamanders, they agreed that that very little was actually known about how salamanders might respond to projected changes in weather conditions, the effects of endocrine disrupting chemicals or diseases such as chytridiomycosis. This uncertainty makes these issues priority research areas.

Pro-active conservation actions were identified for the region, including: 1) Mapping species distributions to identify high-conservation value land for salamander conservation; 2) Improving management of invasive species and diseases; 3) Managing species in captive breeding and conducting translocation experiments to restore extirpated populations of salamanders and manage dispersal issues associated with climate change; 4) Educating local residents to highlight how important and unique salamanders are and identifying ways in which they can change their own behavior to help salamanders; 5) Improving legislation to encourage responsible use of agrochemicals and endocrine disrupting chemicals, restricting residential development in high-conservation value land, mitigating climate change and reducing the extent and impact of mountain-top removal mining; 6) Engaging with other organizations such as Partners in Amphibian and Reptile Conservation (PARC) as well as potential funding partners and researchers to build capacity and interest in salamander conservation.

Please contact gratwickeb@si.edu for more information or subscribe to the Appalachian Salamander Conservation List Serve:  http://lists.aza.org/cgi-bin/mailman/listinfo/appalachiansalamanders
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The Appalachian region of the United States is home to 14% of the world’s 535 salamander species (Appendix 1, Stuart et al. 2004), with very high basal diversity making it an extraordinary salamander biodiversity hotspot (Chippindale et al. 2004; Kozak et al. 2005; Young et al. 2004). Of the 76 salamanders occurring in the Appalachian region (left); nearly half (35 species) are endemic (Appendix 1).

One of the reasons for uncertainty over the status of salamanders in the region is because estimating salamander numbers is a difficult task. Even data collected over long time periods using similar sampling protocols may not function reliably as an index of relative abundance because detection probabilities for these species may vary with weather conditions. Only recently have statistical methods been developed that can account for this variance (Bailey et al. 2004).

Nevertheless, experienced salamander biologists have noted cause for concern. One example from the Appalachian region is a series of numerous long-term studies conducted by Dr. Richard Highton, which suggested that populations of thirty-eight species of Plethodon salamanders may have declined by 50% in the 1990’s and the causes remain unknown (Highton 2005). Salamanders are a unique feature of America’s biodiversity heritage, and out of the 171 species occurring in the USA, the U.S. Fish and Wildlife Service (FWS) lists 13 of these as threatened or endangered (FWS 2008), while the IUCN lists 41 American species as endangered, threatened or vulnerable (IUCN et al. 2006). Given the uncertainty of the status of many salamanders it is clear that a systematic examination of the potential threats to salamanders is needed and some proactive conservation steps are in order.
WORKSHOP OVERVIEW

The Appalachian Salamander Conservation Workshop was co-hosted by the Smithsonian’s National Zoological Park and IUCN’s Conservation Breeding Specialist Group. We assembled a multi-disciplinary team of 35 experts representing state and federal agencies, zoos, universities and non-profit organizations (Appendix 2) to systematically examine the potential threats to salamanders in the Appalachian region, prioritize them and identify potential conservation actions that could be taken to mitigate those threats.

We used the IUCN - Conservation Measures Partnership (CMP) classification of direct threats (Salafsky et al. 2008) to structure 15–20 min presentations by experts to define the scope of each problem in the region. Participants were asked to prioritize threats for Appalachian salamanders (Table 1). The meeting was facilitated by Onnie Byers of the IUCN Conservation Breeding Specialist Group.

Table 1: IUCN Direct Threat Categories Prioritization

<table>
<thead>
<tr>
<th>Rank</th>
<th>Threat</th>
<th>Votes</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Climate Change and Severe Weather</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>Pollution</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Residential &amp; Commercial Development</td>
<td>15</td>
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<td>4</td>
<td>Energy Production &amp; Mining</td>
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<td>5</td>
<td>Invasive and Other Problematic Species and Genes</td>
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<td>Transport and Service Corridors</td>
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<td>8</td>
<td>Agriculture &amp; Aquaculture</td>
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On the basis of this initial prioritization, three working groups were formed to draw concept models for each threat and to identify possible conservation interventions and discuss future research needs. Group 1 - Residential development, Natural system modification, and Transport and service corridors, Group 2 - Energy production, and Climate change, Group 3 - Invasive and problem species, and Pollution

The working groups broadly followed workshop methods and protocols outlined in the IUCN-CMP open standards for conservation document (CMP 2007).

Once concept maps were drafted, they were presented to the larger group for further input and finalization. Group members identified some immediate tasks and deadlines needing attention and volunteered to address them in order to initiate conservation interventions that had been identified.
1. CLIMATE CHANGE

**INTRODUCTION by John Maerz**

Global climate change is the backdrop upon which all other factors influencing wildlife conservation play out. Climate change is globally driven and acts at a scale that presents limited opportunities for intervention in the near future. Though widely recognized as a threat, there remains limited empirical data on the potential impact of climate change scenarios on amphibian conservation. This is particularly true for Appalachian salamanders, which are a significant component of the world’s salamander diversity.

Though the Appalachian region was relatively cool over the past 100 years, 6 of the 10 warmest summers in southern Appalachia occurred within the past 25 years, and extensive droughts occurred from 1984-1987, 1997-1998, 2003, 2005 and 2007 (Lu et al. 2005). Consensus among global climate models (GCMs) predicts a 2-6°C increase in mean annual temperature over the next century for the Appalachian region (Anon 2008). Less appreciated but perhaps more important, more variable temperatures are expected in association with rising mean temperature. GCMs are more variable with respect to predicted changes in precipitation in the Appalachian region ranging from an increase of 10% to a decline of 30%. There is consensus that warming will shift precipitation to one of less frequent, more intense rain events with more extensive rainless intervals and more frequent drought. Most projected climate scenarios are not predicted to cause major biome shifts within the Appalachian region with the exception of a near complete loss of the sugar maple-beech forest replaced by oak-hickory forests (Hansen et al. 2001).

Current evidence of amphibian responses to climate change are limited largely to examples of altered phenology [timing of breeding] (Beebee & Griffiths 2005). These studies show a general trend of earlier breeding consistent with a warming climate. With regard to Appalachian salamanders, a recently published study by (Gibbs & Karraker 2006) indicates a range wide increase in frequency of a warmer adapted, stripe-less color morph of red-backed salamander (*Plethodon cinereus*) over the past century. This is the first published evidence of a potential evolutionary response to climate warming. Another interesting study found that montane adapted species at lower altitudes regularly undergo warming-induced stress (Bernardo & Spotila 2006) and the prognosis is sobering for these species as climates warm and mountain animals retreat further up mountain slopes (Bernardo et al. 2007; Bernardo & Spotila 2006; Parmesan & Yohe 2003). The published literature on Appalachian salamander responses to climate change is otherwise depauperate.

We already know a great deal about the effects of temperature and moisture on the ecology of Appalachian salamanders (particularly the Plethodontidae), so can make some well-informed predictions about the direct effects of projected climate change (Bobka et al. 1981). The activities of salamanders are governed by moisture, particularly rain events. A rise in temperature and less frequent rain events will both increase hydric constraints on salamanders. Shorter and less frequent opportunities to forage and search for mates will likely result in local reductions of salamander abundance. Further, a
significant amount of Appalachian “cryptic” species diversity is linked to high elevation endemics, and rising temperatures may lead to upslope range contractions and local extirpations or extinctions.

Hydrologic changes, particularly as they interact with increasing exurban development in the Appalachian region, may also threaten particular Appalachian salamander groups. Predictions are for lower basal stream flow and more frequent droughts that could threaten stream-breeding species, particularly those with multi-annual larval phases. Heavier pulse rain events with increasing land development are expected to increase pollution inputs into streams and increase scour events that may displace eggs or larvae (similar risks have been identified for stream fishes (Clark et al. 2001)). Finally, warmer temperatures are expected to reduce snow pack, which is responsible for filling isolated wetlands and vernal pools during spring melt. While adapted to the ephemeral nature of these habitats, pool breeding salamanders may be at risk from reduced snow pack.

A greater challenge is our poor understanding of the more complex risks climate change poses for Appalachian salamanders. For example, recent research suggests an important relationship between climate, immuno-competence and disease. Recent evidence from the well publicized extirpations of tropical frogs suggests that rising temperatures and, more importantly, more variable temperatures is a driver of chytrid driven extirpations (J. Rohr, University of South Florida, unpublished data). Additional recent research on temperate salamanders shows that amphibian immune systems can be slow to adjust to temperature changes, particularly sudden drops in temperature (Raffel et al. 2006). The consequence is that as temperatures vary more widely with rising mean temperatures, amphibian immune systems are operating sub-optimally for a greater proportion of the time. This also creates a paradox whereby amphibian populations may be more vulnerable to cool-temperature associated diseases such as chytrid despite rising mean temperatures. How forests responses to climate change will alter terrestrial and fresh water food webs is another complex issue that has not been integrated into projected impacts on wildlife.

Unlike other proximate threats to salamander conservation (e.g., pollution or habitat loss), climate change present few opportunities to alleviate the threat. Rather, management options for contending with climate change must focus on conservation of the ecological and evolutionary potential for salamander populations to respond to changing climates. Evidence that species may have some evolutionary potential to respond to climate warming highlights the need to conserve current diversity both among and within species. Identification of functional diversity within species should become a greater research priority, particularly of museums and zoological programs. Protection of migration corridors will facilitate northward migration of some species, though it will probably be necessary to consider translocation of species such as high elevation endemics. The science of translocations has been neglected for most salamander species [though there are examples of accidental successes]. It will also be important to begin broader efforts at captive husbandry to and “insurance” archiving of at risk species.
**DISCUSSION**

The root cause of climate change is increasing global demands for energy leading to the burning of fossil fuels that increase CO₂ and other greenhouse gases in the atmosphere. In order to mitigate climate change, we need to reduce global use of fossil fuels. However, the actual effects of climate change on salamanders are relatively unknown and we urgently needed to translate regional climate predictions to local-level changes in salamander biodiversity. It is not beyond the mandate of local groups to tackle global climate change, but for practical reasons members of this group should focus on research to determine effects and emergency mitigation measures for species most at risk.

**Shifting species ranges, Altered hydrology & Snowmelt timing and quantity**

Gradually increasing mean annual temperatures would almost certainly lead to shifting species ranges. This would create opportunities for generalist amphibian species such as bullfrogs that would extend their ranges, but lead to range contractions of some restricted-range specialists. Mountain-top endemic *Plethodon* salamanders are already isolated at the very extremes of their natural climatic distribution and would be particularly vulnerable to climatic changes. Even widely distributed salamander species may be negatively impacted by range reduction or expansion of ranges of competitors, predators and changes in forest habitats. These shifting climatic envelopes would also affect species abilities to disburse through habitats that are already very fragmented.

Species that are dependent upon vernal pools created by snowmelt may be disrupted if predictions of less snow with earlier melting times prove correct. Furthermore, climatic models predict that temperature will become more variable, with greater extremes and
that rainfall will become more intense with greater dry spells in between, leading to altered stream hydrology.

Potential conservation actions:
1. Transplanting/relocating species to potentially suitable habitat and providing economic incentives for landowners to manage land for these species (e.g. gopher tortoise). This would require a detailed review of case-studies where there have been successful introductions such as _Plethodon montanus_ at Mountain Lake Biological Research Station, Virginia. Additional experiments with translocation are needed to determine optimal methods such as the comparative benefits of using neonates vs. adults etc. Priorities for experimental translocation would be places where there have been local extirpations of salamanders such as those sites identified by (Highton 2005).
2. Captive breeding for assurance colonies and head starting programs. (e.g. the Wilds conservation center in Ohio is starting an in-stream Hellbender propagation facility).
3. Cryobanking and/or ex-situ breeding for species preservation (e.g. San Diego Zoo has good system for long-term cryopreservation of material).
4. Conducting a “GAP” analysis and determine which species are most at risk to changing climatic envelopes.
5. Researching polymorphism and other specific traits that might have adaptive significance (e.g. red-backed vs. lead backed _P. cinereus_ frequencies).
6. Developing an education campaign using salamanders as a vulnerable “backyard” assemblage of animals that is at risk of extinction from climate change. This will help people to connect climate risk to local animals, not just polar bears. Ecosystem services values should be used to highlight why salamanders matter.

Changes in forest productivity
There is evidence to suggest that increasing CO₂ concentrations increases the rate of growth of trees, but also reduces the nutrient quality of the leaf litter. This has been linked to reduced amphibian productivity in streams. How poor forest fertility might be counterbalanced with high use of fertilizers in agricultural areas is unclear.

Task list

Husbandry – Need to prioritize taxa at risk from climate change and in need of for ex-situ conservation by January 2009 and engage partners willing to initiate research populations to improve understanding of ex-situ husbandry and breeding. Existing knowledge in the community could be captured by requesting literature in a note published in *Herpetological Review* (December 2008). Lead – Andy Odum and Tim Herman.

**Funding** – Identify which academic institutions, state agencies and foundations are interested in providing assistance for amphibian conservation. *Lead - Jennifer Sevin.*

**Cryopreservation** – Need to get tissue banking protocols and find out who may be willing to assist from Ollie Ryder at San Diego Zoo, Andy Kouba at Memphis Zoo and Cincinnati Zoo (task completed, see Appendix 3). *Lead – Andy Odum.*
2. POLLUTION

OVERVIEW by Donald W. Sparling

Compared to other vertebrate classes, there have been very few scientific papers written on the responses of amphibians to contaminants (Sparling 2000). Further, of the 500–600 papers published on amphibian ecotoxicology in the past 10 years, less than 100 included salamanders. Researcher attention has focused on only a few contaminant classes such as pesticides, heavy metals and ultraviolet radiation. Thus our level of knowledge on the effects and tissue concentrations of the plethora of contaminants in salamanders is very limited.

Because more is known about the effects of contaminants on anuran larvae (especially Ranids) than on other groups of amphibians, some extrapolation of findings from this group may be applicable to salamanders. However, there are major factors that hinder extensive extrapolation. First, all groups of animals show some intra- and interspecific variation in responses. Even the computation of the standard measure of toxicity, the median lethal concentration of LC₅₀, acknowledges that there is variation in sensitivity among individuals of the same species. Among species within the same taxonomic family it is not unusual to find a 5 to 10-fold or more difference in LC₅₀ values for a given toxicant; greater variation in response generally occurs with as relatedness decreases. Second, there are important differences among amphibian groups with regards to potential exposure to contaminants. Exposure through aquatic environments continues to receive the greatest attention by scientists. Thus exposure scenarios for aquatic life forms of amphibians such as anuran larvae are better understood than for terrestrial life stages. Predictions of exposure are less precise for salamander species that spend a majority of their life cycles on land such as Ambystomids or Plethodontids than species that are mostly aquatic. Third, the process of metamorphosis is a very critical stage in the life history of anurans. Within a few days an animal is totally transformed from a fish-like, aquatic, detritivore to a terrestrial insectivore with all of the anatomical and neural changes commensurate with these changes. Contaminants that were slowly sequestered in liver or tail during the tadpole stage may be rapidly mobilized to produce effects that would not be apparent in salamanders with no or partial metamorphosis. Unfortunately, we lack sufficient data to determine if there are any consistent differences between anurans and urodeles in their response to contaminants.

With the exception of garbage and solid wastes, all of the IUCN-CMP direct threat categories present risk to salamanders (Salafsky et al. 2008). Domestic sources contain pesticides and many pharmaceutical products that can affect salamander endocrine systems or other physiological functions. Agriculture and forestry practices release pesticides, fertilizers and sediments that may cause direct or indirect harm. Industrial and military sources release a variety of organic contaminants and heavy metals. Many of the contaminants from all sources may become airborne and dispersed to remote aquatic and terrestrial sites where they can have serious impact. In conclusion, we know that contaminants are directly related to amphibian population declines of anurans but we lack vital data on their effects on salamanders.
DISCUSSION

Pollution is a problem to salamanders in sublethal doses that may affect salamander prey base, or disrupt the salamander’s lifecycle by affecting reproduction, immunity, longevity, development or metamorphosis or disruption of the endocrine system. Specific habitats such as streams, pools or land each face very different kinds of contaminants issues. Stakeholders linked to agriculture, and potentially to agricultural pollution include farmers; chemical manufacturers; government agencies such as USDA; EPA; and state counterparts; extension services; consumers of pesticides; and agricultural consumers.
Agricultural and forestry effluents
Compared to historical levels, agriculture and forestry have been on the decline in the Appalachian region. However, those areas that are farmed are faced with pressures to increase production levels and lower costs in order to remain viable, leading to increased use of agrochemicals. Overuse of agricultural pesticides may be attributed to several factors: 1) Misuse of pesticides by farmers; 2) Pests become more resistant to pesticides requiring increased dosage levels; 3) Some crop insurance schemes require farmers to use pesticides in order to be insured; and 4) Farming practices are changing from smaller-scale family farms to larger-scale agribusinesses. We know that these factors are important ecotoxicology issues, but their effects on salamanders are virtually unknown.

In addition to pesticide use, fertilizers are recognized as important issues in many Appalachian catchments such as the Chesapeake Bay. Nitrates from fertilizers and ammonia from feedlots may be directly toxic to several species of stream salamanders. Eutrophication of streams from feedlots and fertilizer use may increase biological oxygen demand in streams and smother important substrates that are important for some aquatic species. Additionally, concentrated animal feeding operations (CAFOs) are often point sources for release of endocrine-active chemicals (e.g., androgens and estrogens) to which effects on salamanders are virtually unknown. Other potentially important factors that should be considered in these systems are the additive effects of multiple stressors, UV exposure, and exposure to polycyclic aromatic hydrocarbons (PAHs) and heavy metals. Existing practices of integrated pest management and organic farming are clear opportunities to influence the system in a positive direction.

Potential conservation actions:
1. The government can provide incentives through the farm bill for integrated pest management, organic farming and sustainable farming.
2. Crop insurance policies now require farmers to use chemicals to reduce pests. The policies need to be modified so that farmers can use alternative forms of crop protection.
3. Encourage through education and other methods an increase in demand for products that are grown with minimal use of agrochemicals – something that may not be as rigorous as truly organic e.g. ‘blue angel’ in Europe.
4. Conduct research on the effects of agrochemicals on salamander species of concern and use the information to educate the regulatory community.

Endocrine disruptors
Endocrine disruptors are chemicals that mimic natural hormones or stop the production or function of hormones, and often interfere with an organism’s development. Pharmaceutical and personal care products may enter waste water systems while several pesticides and some industrial chemicals such as polychlorinated biphenyls (PCB’s) and polychlorinated dibenzo-p-dioxin (PCDD’s) are known endocrine disruptors. Current sewage and water treatment processes do not test for or adequately remove endocrine disruptors, and there is little regulatory oversight in part because it is not clear which federal or state agencies are responsible for regulating this class of pollutants (C. Grimm
pers. comm.). Some work has been done on fishes and frogs, but virtually nothing is known about the effects of endocrine disruptors on native salamanders.

*Potential conservation actions:*
1. Identify chemicals that are endocrine disruptors and determine ‘safe’ concentrations of these products to salamanders.
2. Improve water treatment technology to increase removal of endocrine disruptors and related chemicals.
3. Form an inter-agency taskforce to get federal and state agencies to provide regulatory oversight of endocrine disruptors and pharmaceuticals. Aspects of this issue may already have been addressed – that of an inter-agency taskforce/working group - according to EPA’s Web site (http://www.epa.gov/edrlupvx/edrifact.html).
4. Develop a program for safe disposal of personal hygiene and pharmaceuticals.
5. Partner with other groups already concerned with this issue. There are a number of public health concerns (links to cancer and decreases in human sperm counts) that suggest that folks might have already mobilized around the topic and may have made headway.

**Airborne Pollution**

Acid rain is a result of increased energy demand on the East coast of the USA, linked to the burning of fossil fuels such as coal that release oxides of Nitrogen NOx and SO4 into atmosphere. It has affected 12-14% of Appalachian streams and impacted high-elevation spruce habitats in the Appalachian region.

*Potential conservation actions:*
1. Clean Air Act is currently reducing Sulfur dioxide (SO2) emissions and enforcement should be supported.
2. Increase supply of and demand for clean energy.

**Task list**

*Develop a white paper* – compile information and research needs as appropriate to inform the regulatory community on pollution and effects on salamanders; this could be used as the foundation for a research proposal that overlays pollution variables with landscape maps. *Leads: Christiana Grim, EPA, Don Sparling, University of Southern Illinois and Joel Snodgrass Townson University.*

*Investigate current incentive programs for reducing pesticide use.* Task complete – see Appendix 5 p36 *Lead: Joe Milmoe, Fish and Wildlife Service*

*General education on salamander biodiversity* – this is a crosscutting need for salamander conservation (all threats). Need to investigate how salamanders are presented to visitors in National Parks and Wildlife Refuges in the region. *Leads: Miles Roberts and Marshall Jones, Smithsonian*
3. RESIDENTIAL DEVELOPMENT

OVERVIEW by Joel W. Snodgrass
In the Appalachian Region the extensive deforestation of the earth in the 20th century was followed by an increase in human population size and increasing urbanization (Harden 2004). These patterns are illustrated by the dominance of land use change by urbanization and an increase in the density of human structures in portions of the Appalachian Region (Turner et al. 2003); these trends are expected to continue.

Residential developments and urbanization in general, are linked to many other stressors through similar direct and indirect effects. For example, residential development produces habitat loss and fragmentation, as do other human land use activities such as energy production, mining, and transportation. Indirect effects include habitat degradation in remaining forested areas, including pollution, changes in ecosystem structure and function, and introduction of mesopredators and potentially disease. Here I specifically consider two examples of indirect effects, road salt contamination of aquatic habitats and changes in hydrology and degradation of stream habitat associated with impervious surfaces.

The more insidious effects of residential development are indirect through the introduction of pollutants and degradation of remaining forest patches and their associated aquatic habitats. As an example of habitat degradation, runoff from roads and other impervious surfaces associated with suburban development results in changes in stream hydrology, channel morphology, and ultimate physical habitat conditions within and adjacent to streams (Paul 2001).

Pollutants that accumulate on impervious surfaces are quickly moved to streams and wetlands. Road salts are a prime example and their use is likely to increase with residential development (Kaushal 2005). Road salts can be highly toxic to embryonic and larval salamanders (Karraker 2008) but little information exists on toxicity to life stage of most salamanders.

As a final note, it is important to consider the influences of residential development over multiple spatial scales. For example, streamside salamander abundance is best predicted by land use patterns over the entire watershed (Willson 2003). Therefore, simple solutions such as storm water management ponds and riparian buffers will not substitute for comprehensive land planning at the watershed scale.
DISCUSSION

Terrestrial Habitat Loss and Hydrological Modification

All four indirect threats: roads, residential development, timber harvest, and dams/weirs, were related to habitat modification. Habitat modification affects different species in various ways. Thus, we determined that we must identify the sensitive species and habitat areas in the Appalachians as the first step in a monitoring initiative. This will help to illustrate the gaps in our knowledge and will help to drive efforts to collaborate and proactively influence decision-makers (e.g., developers, land managers, local/state/federal governments). We must also engage partners, and work through existing networks such as Partners in Amphibians and Reptile Conservation (PARC) and the Amphibian Research and Monitoring Initiative (ARMI).

One existing effort is PARC’s “Important Herp Areas”. This group could assist by both nominating important Appalachian salamander areas based on the PARC criteria and implementing management actions in specific areas identified in partnership with the North East and South East PARC regions. In order to engage states, we need to review State Wildlife Action Plans and engage agency directors through the Association of Fish and Wildlife Agencies’ Amphibian & Reptile Subcommittee in a way that can influence policy at the state level.

One effective example of how “smart residential development” has been achieved through designation of important wildlife areas is the Hudson River Valley example http://www.gap.uidaho.edu/Bulletins/13/smith.htm.
Potential conservation actions:
1. Prioritize areas of importance for salamander conservation and engage urban planners, developers and policy-makers to ensure ‘eco-friendly’ development.
2. Educate landowners about things they can do to stem pollution, landscape appropriately and manage storm water within their watersheds.
3. Control mesopredator populations through removal, or improved garbage management.

TASK LIST

Mapping priority areas - there are several resources available, such as The Nature Conservancy and other sources for habitat information in GIS format, and the species data can be queried through the Lannoo et al. county map data (Lanoo 2005) as well as through key people who can provide the appropriate information. The goal will be to analyze and compile these data into an Appalachian-species specific map as defined by an Appalachian-specific ecoregions by June 2009. Lead - Bill Peterman and Lannoo lab

Establish a clear basis for taxonomic decisions – The current amphibian taxonomy has several deficiencies that result in inconsistent nomenclature. We need to decide which taxonomic authority we should be subscribing to e.g. the Amphibian Tree of Life (Frost et al. 2006). Lead - John Crawford

Compile available habitat cover layers - keeping in mind the appropriate fine scale for the Appalachians. Lead - SEEKING ASSISTANCE

Nominate Important Herp Areas through PARC by June 2010 Lead - Bill Peterman

Create and pilot a standard salamander monitoring protocol - in partnership with herpetology labs across the region. Standard sampling methods will be devised that review existing methods e.g. (Heyer 1994) and recommend standard protocols for terrestrial and stream salamanders in the Appalachians by end of July 2008. Hellbenders have a lot of attention right now and several folks are working on this already. A draft funding proposal for a wider monitoring network will be compiled and circulated. Lead - John Crawford. Potential partners (e.g. John Maerz, Joe Pechman, James Petranka, ARMI (Larissa Bailey, Susan Walls), Paul Sattler (Liberty Univ.), Jason Gibson (VA Herp Society) will be recruited by Joel Snodgrass. Funding opportunities through the Smithsonian will be explored by Steven Montfort.
4: ENERGY PRODUCTION AND MINING

OVERVIEW by Tom Pauley

The USA extracts over 1 billion tons of coal each year and one third of this is extracted from the Appalachian region (NMA 2007). About 2/3 of all coal in the Appalachians is mined underground and about 1/3 of this is derived from surface mining, primarily through mountain top-removal (NMA 2007).

Of all the mining practices in the Appalachians, mountain top removal is the most visible and destructive for salamanders. For example, in 2007, West Virginia alone had 300,000 acres of active mountain-top removal mining permits amounting to 2% of the State’s total land area (Anon. 2007). Salamanders are the vertebrates hardest hit by these activities and their populations can take as long as 70 years to recover to pre-disturbance levels (Williams 2003). The flattened topography enables alternate land uses of restored areas, and alters the soil structure and hydrology of reclaimed areas, while filling valleys with overburden has been shown to cause sedimentation downstream that may negatively affect stream salamanders (Starnes & Gasper 1995).

Strip mines are not as prevalent as they used to be, but now there are a lot of abandoned strip mines, and it is unclear who should be responsible for their restoration. Strip mines often alter hydrology in a way that leads to drying on mountain tops that will negatively affect *Plethodon* species, but ponds created near the mining activities themselves act like vernal pools, facilitating species such as wood frogs and *Ambystoma* that would not ordinarily survive without these bodies of water.

Acid mine drainage is the biggest problem associated with deep-mining, and has been mitigated by dumping limestone in affected streams (Middlekoop et al. 1999). However, mineshafts can be used by some species of salamanders if they are not too acidic and may actually facilitate some species (Pauley and Pauley 2007).

Road construction is another issue associated with mining and with wind farms. Rights-of-way lead to forest fragmentation. And once established, roads create ecological edge effects that reach up to 100m into forest floors, changing microclimate and providing access to edge predators such as snakes, cowbirds, blue jays, and turkeys.
DISCUSSION

The primary driving force for mining in the Appalachian region is a growing domestic market for electricity and widespread political and social desire to reduce dependency on foreign energy. The potential for converting coal to gasoline is a factor that could greatly increase demand for coal in the future. The need to create jobs in this impoverished area of the country often undermines any political will needed to create environmentally stringent regulations that might hurt the coal industry.

Mountain top removal
Mountain top removal will impact terrestrial salamanders, including: high elevation endemics, low elevation cosmopolitan species, low elevation specialist species, stream salamanders and cave-dwelling salamanders. However, the aquatic species are the most vulnerable to this kind of disturbance. As mountain topography is altered, high-elevation habitat is lost and flattened land offers opportunities for infrastructural development in
reclaimed areas. Valley-filling activities result in flooding, sedimentation, aquatic pollution and alter organic matter, cover and the prey base.

**Potential conservation actions:**

1. Engage mining companies – biologists should form partnerships with mining companies and coal associations to secure access to sites, document impacts and encourage scientifically sound long-term monitoring and environmental mitigation plans, and encourage them to find alternative methods for disposing of displaced fill. Environmental impact assessments and mitigation plans should be reviewed by independent scientists for their quality.

2. “No net loss” schemes such as those devised for wetlands should be encouraged. Mountain-top removal should be limited to low-conservation priority areas and high conservation priority reserves should be set aside as part of mitigation plans.

3. An inventory of priority conservation sites and species should be conducted and shared with stakeholders, including mining companies, states, and federal agencies who could incorporate the information into their planning processes.

4. Improve legislation by engaging other environmental groups currently lobbying for improved regulation of the mining industry. Provide them and congressmen such as Nick Rahall (WV) with information on salamander conservation that may help to strengthen arguments for improved regulation of mountain-top removal.

5. Increase consumer demand for alternative fuels and encourage use of energy efficient devices that will lead to reduced demand for coal. This should be done by engaging in dialogue with existing conservation groups that can educate their members in the Appalachian region.

6. Enforcement of existing laws should be investigated to ensure that existing practices are in compliance with the Clean Water Act. Any fines should go towards mitigation funds that improve salamander conservation in the region.

7. Incentives such as tax breaks could be suggested for less damaging underground mining methods.

**Strip Mining**

Strip mining has resulted in girded mountain islands that permanently fragment and isolate mountain-top habitat and alter the hydrology of these systems leading to the drying out of forest fragments and severe edge effects. The girded pools associated with abandoned strip-mines for species that are not normally found at these sites, compromising the original species assemblage.

**Deep Mining**

Deep mining has resulted in point-source pollution from slurry ponds and acid-mine drainage issues. Deep mines can alter local hydrology and the infrastructure such as roads, slurry ponds and buildings that service the mines impacts salamander habitats directly.

**TASK LIST**

Lead - Robin Saunders, NZP. Compile regulatory information in regards to mountain top mining in Appalachia by early July. Lead – Marshall Jones, NZP.


Inform other organizations - contact Defenders of Wildlife and encourage support for this issue as an advocacy organization by early June. Lead - George Rabb, Brookfield Zoo. Circulate one-page summary of the white paper to other organizations such as the Piedmont Environmental Council by late August. Lead - Marshall Jones, NZP.

Increase awareness – Based on white paper, educate others about the relationships between mountain top removal and amphibian conservation. Lead – Shelly Grow; AZA.
5. INVASIVE AND OTHER PROBLEMATIC SPECIES

OVERVIEW by Rob Brucker

There are many invasive and problematic species that have been established in the eastern United States, all of which are attributed to anthropogenic movement. Some studies have found correlations between native Appalachian salamander populations and how the introduced species are impacting them. Various plants, animals, and diseases that have entered the ecosystems have had widely unknown consequences on amphibian populations and diversity.

Two examples of introduced species that are impacting salamanders are the brown trout (Salmo trutta) and the hemlock woolly adelgid (Adelges tsugae). The brown trout was introduced as a sporting fish that quickly populated ponds, lakes, rivers, and streams throughout North America (Fuller 2008). This opportunistic predator had been found to eat amphibian larvae, reducing the number of larvae that survive through metamorphosis (Heenar & M'Closkey 1997).

The hemlock woolly adelgid is an insect species unintentionally introduced to North America that has caused massive devastation to the hemlock trees found within the North eastern region of the USA. An insect infested tree slowly dies over a period of four to ten years resulting in a loss of canopy cover and leaf litter that supports terrestrial salamander’s territory (Mathewson 2007). The change in plant diversity and cover results in microclimate changes within the ecosystem that makes the ground temperatures too warm and less humid to maintain salamander populations.

One potential threat to the Appalachian salamander species is the fungal pathogen Batrachochytridium dendrobatidis (Bd). This disease has been implicated in the declines of amphibian species around the globe but little is known about how it is impacting the salamanders of the Appalachians. Salamanders are often perceived as being resistant or immune to Bd and thus overlooked in regards to research. The majority of current research focuses on anuran responses to the pathogen and does not include systematic observations on the susceptibility of different salamander species. It is known that several species of salamanders of the region (The Redback salamander Plethodon cinereus, the Fourtoed salamander Hemidactylium scutatum, and Hellbenders Cryptobranchus alleganiensis) have tested positive for the pathogen in the wild. Of these species, the stream dwelling Hellbender is the only species that has been found to succumb to the disease though the cause of death may not be directly due to and infection of Bd (Briggler 2007).

A complete explanation for the variation seen in salamander resistance to the disease has yet to be described. One hypothesis is that the microsymbionts living on the skin of salamanders inhibit the growth of the pathogen (Harris et al. 2006). Though it is unknown if all salamanders within the region are affected by Bd, the exploitation of beneficial bacteria may provide long-term resistance to B. dendrobatidis for vulnerable amphibians and aid in conservation efforts within Appalachia and elsewhere.
DISCUSSION

Disease and Pathogens
Very little is actually known about diseases in salamanders, their natural occurrence and incidence rate or the extent or susceptibility of different salamander species to *Bd*. We do know that potential vectors of disease are from: amphibian trade for pets, research and food; and translocation of disease by researchers sampling between ponds. There is also a possibility that climate change and other environmental stressors such as pollution may increase the susceptibility of amphibians to diseases, but this is mostly unknown.

Potential conservation actions:
1. Identify critical information gaps with regards to salamanders and diseases and pathogens, distribution and vectors and begin research to improve understanding of disease distributions and ‘at risk’ species. Strategy should proactively identify new pathogens that we can manage before they spread too widely.
2. Increase regulations on intrastate and interstate transport of salamanders.
3. Encourage federal and state agencies to recognize salamanders as wildlife with all the protections therein.
4. Strengthen regulations and increase awareness in the pet trade, emphasizing quarantine protocols, and testing for diseases. May want to work with Pet
Industry Joint Advisory Council (PIJAC), since they are developing a Bd-free ‘Phibs campaign (http://www.pijac.org/i4a/pages/index.cfm?pageid=416). The Web site should be completed soon, according to Jamie Reaser, PIJAC.

5. Increase public awareness of disease issues through web-based outreach campaigns through AZA institutions.

6. Develop standard protocols to document population die off and diagnosis of causes. Should connect with Purnima P. Govindarajulu who spoke on “Field-based protocols for minimizing Bd transmission and monitoring for disease outbreaks” at PARC’s Bd workshop. There was also a working group dedicated to coming up with some of these protocols and updates are available through the PARC Web site (http://www.parcplace.org/Bd_conference.html).

Invasive Flora and Fauna
We have a fairly good idea about where the invasive fish species are distributed in this country see “State of the Nation’s Ecosystems Report”. Many anglers introduced non-native trout species into streams that did not have top predators. Many trout have been introduced under a conservation flag, but they will surely prey on aquatic salamanders and negatively impact their populations. This is good example of the “unintended consequences” of poorly planned conservation actions. In addition to non-native predator fish introductions anglers may use salamanders for bait and unwittingly translocate animals (and diseases) between watersheds.

Not many people realize that most earthworms are an introduced species in North America, and they can drastically affect the amount of standing leaf litter in a forest and therefore impact prime terrestrial salamander habitat; however, there is no effective management strategy available for earthworms at this time.

Some native browsers such as deer and mesopredators like raccoons and opossums have increased dramatically with the decline of sport-hunting and the historical extermination of natural top predators such as cougars and wolves at the turn of the last century. These are likely to be negatively affecting salamander populations either directly as prey animals or their leaf-litter habitat will be negatively impacted by high browser densities.

Potential conservation actions:
1. Increase public awareness of the threats from invasive species.
2. Encourage use of native and endemic species of plants for landscaping purposes.
3. Reduce transport of exotic earthworms from one site to another. Also encourage anglers to kill their bait (or return it to the bait shop) and not return it to the wild.
4. Engage angling groups such as Trout Unlimited and watershed groups like River Keeper to educate stakeholders.
5. Discourage stocking of brown trout and encourage catch and keep on large exotic brown and rainbow trout, but catch and release of smaller, native brook trout.
6. Encourage bait dealers to buy bait back from anglers to decrease the spread of exotic salamanders.
7. Form a salamander enthusiast conservation group “Salamanders unlimited”.

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**TASK LIST**

**Education:** Partner with PARC to facilitate salamander education in the Appalachian region. Specific campaigns could be targeted at stakeholder groups such as pet trade participants or trout fishermen identified in logic model. *Lead: Brian Gratwicke, Smithsonian.*

**Salamander Pathology Center:** Investigate the feasibility of implementing an Appalachian Pathology Center/Clearing House. Develop a network with Appalachian Conservation groups so that samples are sent to a central area for diagnosis and record keeping. This would require a source of funding and extensive outreach to states and biologists. The purpose of this would be to monitor and diagnosis die-offs of salamanders; possible toxicology issues, surveillance for new pathogens; survey distribution of salamander pathogens; and serve as liaison among agencies. Initial steps would be to develop a collection and sampling protocols or to modify existing National Wildlife Health Center protocols. Part of this could also include identifying pathologists who would be willing to provide testing or diagnostics for free or a minimal charge to sample providers. Develop or use of a salamander disease listserve. *Lead: Tim Walsh, Smithsonian.*

**Promoting Use of Native Plant Species in Gardens:** Approach Ellen Gable at the National Fish and Wildlife Foundation to find out how this might fit into the Pulling Together Initiative. *Lead: Brian Gratwicke, Smithsonian.*
6. NATURAL SYSTEMS MODIFICATIONS

OVERVIEW by John (J.D.) Kleopfer

Fire is a natural function in the ecology of many ecosystems and can influence composition, structure and landscape patterns. In the United States, lightning starts more than 6,000 fires annually. Prior to the 1500’s, millions of Native Americans used fire regularly to clear undergrowth and stimulate herbaceous growth. However, fire suppression policies over the past century have greatly affected many fire dependant ecosystems and wildlife found in these places (Smith 2000). Although aquatic ecosystems are not as fire dependant, they will burn during times of extreme drought (Mitchell 2006). When fire is excluded from these habitats, the canopy can become overgrown leading to the disappearance of local amphibian populations (Skelly 1999).

Artificial impoundment of water can have detrimental effects on the aquatic environment. The changes in hydrology, flora and fauna can create uninhabitable conditions for stream dwelling salamanders. The most notable influence of an impounded stream is the accumulation of water and other materials behind the dam. This sequestration limits the downstream flow of nutrients and sediments, which is critical to downstream riparian function. The conversion of a free-flowing stream to an impoundment can change both the flora and fauna of the habitat. The most obvious effect is the loss of riparian habitat and those species adapted to these ecosystems. Native plants and fish are often replaced by nonnative species (Winters 2004).

Few studies have been conducted on the effect of timber harvests on salamanders in the eastern United States. However, it is known that environmental disturbances that modify temperature, humidity or soil moisture will have an effect on most salamander species that inhabit the southern Appalachian Mountains (Petranka et al. 1993). Salamanders will forage less under drier conditions and prey abundance may decrease. In addition, this condition may reduce the quality of the prey via a shift to a more chitinous invertebrate species (Knapp et al. 2003).
7. TRANSPORTATION AND SERVICE CORRIDORS

Overview by Bill Peterman

Roadways, utility lines, and railroads (i.e. service corridors) traverse the landscape, connecting society to goods and services. All of these are common, everyday fixtures on the Appalachian landscape, but impacts to natural systems are largely unknown (Forman & Alexander 1998). It has been estimated that 6.2 million km² of roadways are impacting 15–20% of land and waterways in North America (Forman & Alexander 1998). The short term and long term effects of these service corridors have largely been unstudied, but with increasing concern for salamander conservation, it is imperative that objective evaluations and assessments of these habitats be made in order to make informed management and conservation decisions in the future.

Transportation and service corridors can impact salamanders in a number of ways; all effectively fragment contiguous habitat into smaller patches, exposing core habitat to edge effects and altering the microclimates therein (Forman 2003). Beyond changes to microclimate that can alter salamander abundance and activity (Marsh & Beckman 2004), corridors may serve as dispersal barriers to salamanders (Carr & Fahrig 2001; Marsh & Beckman 2004) or affect populations through increased mortality (Hels & Buchwald 2001).

In addition to fragmenting the landscape, service corridors may expose previously insulated habitats to invasive species and / or disease (Urban 2006). Further, these corridors can impact aquatic systems through increased sedimentation, runoff, or other point source pollution (Gillespie 2002). Construction of corridors may subject wetlands to increased insolation that can alter hydrology and production. A contemporary solution to wetland alteration or destruction is mitigation, but it is not widely accepted as to what mitigation practices work or how to best recreate wetlands (Matthews & Endress 2008).

As a result of these numerous influences to both upland and aquatic habitats, many salamander species are affected, some at multiple life history stages. The Appalachian region harbors a great diversity of endemic and locally distributed species that are of particular conservation concern, all of which are influenced by service corridors in some aspect or another.
8. AGRICULTURAL THREATS TO APPALACHIAN SALAMANDERS

OVERVIEW by John A. Crawford

The Appalachian-Blue Ridge forests ecoregion encompasses major portions of Fenneman’s Blue Ridge and Ridge and Valley physiographic provinces of the central and southern Appalachians. This region stretches north from northeastern Alabama and Georgia, through eastern Tennessee, western North Carolina, Virginia, and Maryland, and into central Pennsylvania (McGinley 2007). Approximately 83% of the habitat in this ecoregion has been altered and the heaviest loss in habitat can be found in the ridge and valley provinces, particularly in limestone valleys that are most productive for agriculture (McGinley 2007).

Agriculture can encompass many different forms such as annual and perennial non-timber crops, wood and pulp plantations, livestock farming and ranching, and aquaculture (Salafsky et al. 2008). In the contiguous United States agricultural land makes up approximately 23% of the total land available, with another 31% devoted to grassland, pasture, and range uses (Lubowski et al. 2002). Within the central Appalachian region, grassland management and pasture-based livestock production account for the majority of agricultural acreage and about 25% of the total regional land use (Buergler 2004). This conversion to and use of agricultural land in the Appalachian region likely has a number of direct and indirect effects on salamander populations. These effects include, but are not limited to, habitat loss, edge effects, and decreased water quality (via increased siltation and chemical run-off).

Whereas the conversion to and use of agricultural land has obvious direct and indirect effects on salamander populations, the historical “footprint” left from abandoned agricultural lands is just as important. During the early 1900’s, local rural economies were primarily agricultural. By the mid-1900’s, the regional economy began to change toward an industrial and service-based economy (Hicks & Pearson 2003; McTammany 2004). This shift away from agriculture resulted in extensive regrowth of forests on abandoned farmlands (McTammany 2004). However, biological and community structure, as well as salamander populations, do not fully recover following reforestation of agricultural lands for at least 50 years (Hicks & Pearson 2003; McTammany 2004). While the conversion of land to agriculture is minimal in the current era, consideration must be given to conservation techniques that will return Appalachian salamanders to pre-agricultural population levels on abandoned farm and agricultural lands that are currently being reforested.
REFERENCES


ACKNOWLEDGEMENTS
We thank John Berry the National Zoological Park’s Director and the Associate Director of Science Steve Monfort, for their strong advocacy for amphibians and for providing the space, facilities and support to host this workshop. Onnie Byers from IUCN’s Conservation Breeding Specialist Group kindly donated her time to facilitate the workshop’s ambitious agenda. Jim Murphy has been the steady advocate for salamander conservation of the working group, helped to convene the experts and proof-read this report. The editor also thanks Dave Wildt who provided guidance needed to host the workshop and to CRC staff, especially Nicole Tarmon, Jennifer Buff, Jennifer Sevin, Laura Walker and Hilda Mauck who assisted significantly with logistics.

Funding for meals and refreshments was provided by the Friends of the National Zoo. Flights for several participants were kindly donated by AirTran. We are grateful to Conservation International for funding the Amphibian Conservation Program at the National Zoo.

About the Smithsonian Amphibian Working Group
The Smithsonian Amphibian Working Group is a cross-unit group of amphibian researchers and associates in the Smithsonian Institute formed in order to form an appropriate institution-wide response to global amphibian declines. The group meets monthly and was established to facilitate communication and coordination of the various amphibian research activities within the Smithsonian.

The working group identified Appalachian salamanders as an assemblage of amphibians in need of conservation attention. The National Zoological Park’s CRC center in Front Royal is a 3,200 acre research facility in the Appalachians and the infrastructure represents significant institutional capacity within the region. The salamander conservation workshop was convened to engage the significant regional expertise as partners and mobilize internal expertise to devote their time and skills to this important conservation issue.

IUCN Conservation Breeding Specialist Group
The Conservation Breeding Specialist Group (CBSG) is a global network of conservation professionals dedicated to saving threatened species by increasing the effectiveness of conservation efforts worldwide. CBSG is recognized and respected for its use of innovative, scientifically sound, collaborative processes that bring together people with diverse perspectives and knowledge to catalyze positive conservation change.

CBSG is part of the Species Survival Commission of the IUCN-the World Conservation Union-and is supported by a non-profit organization incorporated under the name Global Conservation Network. Our ties to the IUCN are essential to the strength of CBSG and its position as a vital link among governments, conservation organizations, and others in the conservation community.
# APPENDIX 1: APPALACHIAN SALAMANDER CHECKLIST

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>AZA Priorities</th>
<th>Nature Serve Status</th>
<th>Endangered Species Act Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ambystoma barbouri</em></td>
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<td>Common Name</td>
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<td>Plethodon cinereus</td>
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<td>Siren intermedia</td>
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* = Appalachian Region Endemic

1 = These numbers are derived from an AZA decision making tool that assigns values to rank those amphibian species that would most benefit from ex situ conservation action. Values for amphibian species in the United States and Canada range from 4-68.

This table was compiled from data available through the NatureServe Website [www.natureserve.org](http://www.natureserve.org) (G1 = most imperiled, G5 = secure), the AZA website [www.aza.org](http://www.aza.org) and FWS website [www.fws.gov/endangered](http://www.fws.gov/endangered)
## APPENDIX 2: LIST OF PARTICIPANTS - SMITHSONIAN APPALACHIAN SALAMANDER CONSERVATION WORKSHOP

<table>
<thead>
<tr>
<th>Last</th>
<th>First</th>
<th>Affiliation</th>
<th>Email</th>
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</thead>
<tbody>
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<td>Jim</td>
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<td>Andy</td>
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<td>Padea</td>
<td>Luis</td>
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<tr>
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<td>Joel</td>
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<td>Sparling</td>
<td>Don</td>
<td>University Southern Illinois</td>
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</tr>
<tr>
<td>Thieme</td>
<td>Michelle</td>
<td>World Wildlife Fund</td>
<td><a href="mailto:michele.thieme@wwfus.org">michele.thieme@wwfus.org</a></td>
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<tr>
<td>Walsh</td>
<td>Tim</td>
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<tr>
<td>Weiss</td>
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<tr>
<td>Wildt</td>
<td>Dave</td>
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<td><a href="mailto:wildtd@si.edu">wildtd@si.edu</a></td>
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APPENDIX 3: PROTOCOL FOR TAKING A POST MORTEM AMPHIBIAN BIOPSY FOR CELL CULTURE

by Conservation and Research for Endangered Species, Zoological Society of San Diego Zoo, Cytogenetics Division, Telephone: (760) 747-8702 ext. 5716

General Principles: The single most important factor in taking a biopsy sample is the proper cleaning of the sample site. Tissue cultures must be sterile; bacteria or fungi kill the cultures. The fresher the sample is, the better it will grow. No antiseptic (such as mercurochrome or ioprep) can be used before biopsy. Only 70% alcohol should be used to cleanse the biopsy site.

Preferred Biopsy Sites
1) Eye (whole eye); 2) Kidney; 3) Skin; 4) Throat; 5) Tongue; 6) Gonads; 7) Tail (juveniles).

Materials Needed
1) 70% alcohol; 2) Sterile gauze; 3) Sterile forceps and scalpel; 4) Biopsy vials with fresh medium (MEM + 10% FBS, + 1% Glutamine/Pen-strep, + 1% fungizone).

Protocol
1. Moisten the biopsy area with alcohol.
2. Use 70% alcohol-drenched gauze to clean the biopsy site very, very well.
3. Get the recipient bottle, containing tissue culture medium ready. Using sterile forceps and scalpel, grasp a piece of the tissue, about the size of a small bean or pea. Place immediately into recipient bottle and close cap tightly.
4. Label bottle with species, ID number, sex, institution, and date.

Shipping
Ship the tissue at room temperature via FedEx or UPS express overnight priority. DO NOT SEND FROZEN. Please notify the lab that the samples are coming. Contact Marlys Houck (760) 291-5454, mhouck@sandiegozoo.org, or Suellen Charter (760) 747-8702, x5716, scharter@sandiegozoo.org.

Ship to: Marlys Houck
San Diego Wild Animal Park- CRES
15600 San Pasqual Valley Road
Escondido, CA  92027-7000
(760 ) 291-5454
APPENDIX 4: CONSERVATION WORKSHOP PARTICIPANT RESPONSES TO INTRODUCTORY QUESTIONS

1. What do you hope will be accomplished during this workshop?
   - Overview of problems facing salamanders and possible solutions – directions what should be taken.
   - A movement for salamander conservation that will spark action in the general public as well as the scientific community.
   - A plan for salamander conservation that will include ex situ component, as appropriate, and will be available for other zoos and aquariums to tap into and/or learn from as a template for use in their own programs.
   - Establish a core group of dedicated people to identify the major threats facing salamanders in the region and to set up the infrastructure/action plans to make long-term differences.
   - Identify the two or three major threats to salamanders in the region. Focus on the direct and indirect effects of these threats
   - A more cohesive, interdisciplinary approach to amphibian conservation.
   - To focus the Smithsonian and amphibian partners on the amphibian conservation work needing to be done locally; to work as a community and not individually; to broaden the interest in education and training and perpetuating amphibian conservation in the long term
   - Besides just identifying problem areas and possible solutions to these problems. I’d like to see the group discuss practical application and how to implement these to help conserve salamander populations long term
   - Produce a report, develop an action plan and then implement it; get an big picture for landscape conservation of salamanders; learn more about threats to salamander biodiversity; meet experts
   - Production of a report that will be effective in stimulating conservation of salamanders and other amphibians. Develop an action plan that will be implemented; enable the release of funding or to identify sources of funding for salamander conservation.
   - Increase my knowledge about salamander biology and existing threats to their survival; develop a realistic set of scientific and conservation goals with well defined action steps, timelines, and identify champions to achieve measurable success.
   - Direction for regional salamander conservation that firmly links all zoos with local/national conservation initiatives, and establishes strong, interdisciplinary collaborations among all interested parties (incl. NGOs like Trout Unlimited and Audubon Society) since they are also interested in forested habitats.
   - Develop relationships that will bind me to the group; learn specific issues of the region as they relate to salamander conservation; maybe see a salamander or two.
   - A set of salamander goals and strategies with a basic outline of steps to achieve them including those related to management.
2. **What do you hope to contribute?**

- What I have learned over 40 years of field work might be used in some way.
- Background information on the biology of Appalachian salamanders
- A better understanding of disease that impacts salamanders and the lack of knowledge therein.
- Reinforce the idea of partnerships so that these discussions and forthcoming work will help bolster and tie into the complement of amphibian conservation work of zoos and aquariums. Offer the support of AZA and our resources.
- A knowledge of salamander ecology, biodiversity, and research/monitoring methods.
- I hope to contribute knowledge pertaining to the mitigation of threats and identify opportunities for restoration.
- Views from the museum research community and the wildlife management community.
- Primarily my enthusiasm, a commitment to continue amphibian research, education and training.
- I would like to contribute my time and energy to help in the field as well through husbandry practices in in situ-ex situ salamander conservation.
- Networking and facilitating larger plan; build alliances and partnerships; formalize a core group; commitment to implementation;
- A perspective on the effects of contaminants and their potential risks to amphibians/salamanders
- I hope to use the results of this workshop to generate interest in salamander conservation that can be translated into tangible resources to support the action steps proposed in this workshop.
- Eventual prioritization of carrying capacity for captive breeding programs and for zoo-based science on basic biology; field work assistance as possible (by me or Animal Care Directorate employees); public awareness of climate change (write and edit children’s books and do community outreach on climate change).
- A broad scale view that influences conservation planning in ways that benefit all wildlife of the region.
- A perspective from both the state wildlife management agencies and from the PARC network with respect to needs/info gaps and conservation tools available (and potentially to be created) to assist in achieving goals set forth here; I also have field experience with stream- and pond-dwelling salamanders in Maryland and Virginia.
APPENDIX 5: AGRICULTURAL AND PESTICIDE USE INCENTIVE PROGRAMS
by Joe Milmoe.

Point source and non-point source contaminants are generally separated between distinct regulatory vs. incentive efforts. Point source are obviously more easily targeted with regulatory and tax measures that prohibit unsafe uses from producers and applicants. Non-point source pollutants are harder to identify, pinpoint, and regulate, so the incentive programs then come into play.

The NGO’s mentioned in the breakout sessions generally have not developed or house their own incentive programs, but rather provide educational material that refers citizens to the federal, state and local programs available. These federal incentive program descriptions are often very vague, allowing for modification on a project-by-project basis. These incentive programs generally combine 1- financial incentive, 2- technical assistance, and 3-education and outreach. Financial incentives are generally small, but considered to be effective when small, inexpensive changes to daily practice are required. Some examples of these federal programs are:

US Fish and Wildlife Service
Partners for Fish and Wildlife Program
The Service’s premier voluntary habitat restoration program, which provides financial and technical assistance to private landowners across the United States. This program targets the 73% of privately owned land in the United States by developing and implementing projects on an individual basis. Projects are “HABITAT” focused, designed to benefit the Federal Trust Species, but regularly incorporate components of sustainable agriculture, riparian buffer zones, decreased pesticide use, etc.
http://www.fws.gov/partners/

USDA NRCS Environmental Quality Incentives Program
A voluntary conservation program for farmers and ranchers that promotes agricultural production and environmental quality as compatible national goals. EQIP offers financial and technical help to assist eligible participants install or implement structural and management practices on eligible agricultural land.
http://www.nrcs.usda.gov/programs/eqip/

USDA Water Quality Incentive Program
U.S. Department of Agriculture’s (USDA) Water Quality Program, is a national effort to encourage the adoption of less polluting farm management practices. Three relevant components of this program are: Education, Financial, and Technical Assistance.

EPA Pesticide Environmental Stewardship Program
A voluntary program that forms partnerships with pesticide users to reduce the potential health and environmental risks associated with pesticide use and implement pollution prevention strategies.
http://www.epa.gov/oppbppd1/pesan/
**EPA Strategic Agricultural Initiative Grants**

The primary goal of the Strategic Agricultural Initiative (SAI) Project is to assist in Food Quality Protection Act implementation though an extensive and effective communication and partnership effort with regional pesticide user, teacher and researcher communities. The SAI provides grant funding for FQPA-related projects for state and nonprofit organizations and seeks partnerships with regulatory agencies.

[http://www.epa.gov/pesticides/grants/aginitiative.htm](http://www.epa.gov/pesticides/grants/aginitiative.htm)

Many additional initiatives exist in a wide variety of Farm Bill incentives, including: easements, contracts, cost sharing programs, integrated pest management, irrigation scheduling, and habitat credit trading.

One notable development in the most recently approved Farm Bill is the Environmental Services Markets (Sec 2709). This deals mainly with carbon sequestration but also relates to a number of agricultural and pesticide uses. The ESM requires 1) the development of a procedure to measure environmental services benefits; 2) a protocol to report environmental services benefits; and 3) a registry to collect, record and maintain the benefits measured.

Many smaller programs and state and local level Departments of Environmental Quality Agriculture, and Fish and Game agencies exist to provide incentives to private landowners and commercial agriculture production.

Finally, I felt the Wildlife Habitat Council ([www.wildlifehc.org](http://www.wildlifehc.org)) to be an important organization to direct further outreach efforts towards. This NGO focuses on corporate land stewardship and is comprised of executives from many industry leaders that were mentioned in the breakouts. WHC utilizes a prestigious habitat accreditation process in which companies strive to become recognized.