Copper Effects on Pond–breeding Amphibians

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Abstract

An artificial wetland was constructed on the Savannah River Site to treat process and storm water discharge from an industrial facility. Constructed wetlands can provide new habitat for local wildlife, but if poor water quality limits recruitment, these wetlands could become "population sinks." In order to assess potential impacts to local pond-breeding amphibians, we are experimentally examining the effects of trace metal exposure on the southern toad (*Bufo terrestris*), the eastern narrowmouth toad (*Gastrophryne carolinensis*), and the southern leopard frog (*Rana sphenocephala*. Cu concentrations generally range from 20-37 ppb and have reached up to 590 ppb in the retention pond; these concentrations may reduce larval survival, increase larval period, decrease body mass at metamorphosis, and cause a reduction in population level genetic variation. Our experiments are ongoing but data to date show that survivorship is strongly affected by Cu treatment. We are now collecting genetic data to assess whether Cu exposure may affect population genetics by causing a reduction in genetic variation and/or alteration of allele and genotype frequencies. These shifts can result from fitness differences among individuals exposed to contaminants or from reduced population size caused by decreased survivorship and reproduction.

1. Background:

- Considerable ecotoxicology research has been conducted on amphibians – most limited to examining the effects of contaminants on egg viability over a short (72h) period
- Little known concerning the effects of contaminants on survival to metamorphosis and size at metamorphosis (key life history traits).
- Almost no genotoxicology research on amphibians
- The importance of examining the genetic impacts of contaminant exposure has been known for decades.
- Most wildlife studies focus on genetic mechanisms of damage to individuals, however, more recently, research emphasis has switched to investigating the population-level impacts in order to predict the long-term effects of contaminant exposure on populations, communities, and ecosystems (reviewed in Belfiore & Anderson 2001).
- Population genetic effects can include an increase in germ-line mutation rates, as in herring gulls (Yauk et al 2000) and mice (Yauk et al 2008) exposed to air pollution.
- Population changes can include reduction in genetic variation and/or alteration of allele and genotype frequencies (Fratini et al 2008, Nowak et al 2009).
- These shifts can result from fitness differences among individuals exposed to contaminants or from genetic drift in small populations (reviewed in Medina et al 2007).
- The effects of contaminant exposure on population-level genetics can be subtle but can have large long-term consequences, making them an important endpoint in ecological risk assessment (van Straalen & Timmermans 2002).

Southern Toad (*B. terrestris*)

2. Project Objectives:

- Document metal concentration in H-02 wetland system
- Determine which species successfully reproduce in the wetlands
- Assess impacts to three local pond-breeding amphibians that differ in larval period and feeding strategies by experimentally examining effects of Cu exposure on:
  - Egg development
  - Larval development
  - Size at metamorphosis
  - Whole body metal burdens in eggs/larvae/metamorphs
  - Patterns of differential gene expression in survivors
  - Changes in genetic variation from starting population → survivors of egg trial → survivors of larval trials
  - Future: Conduct genome association mapping for several traits

Eastern Narrowmouth Toad (*G. carolinensis*)

3. Study Species:

- Southern toad, *Bufo terrestris*
- Eastern Narrowmouth Toad, *Gastrophryne carolinensis*
- Southern Leopard Frog, *Rana sphenocephala*

Southern Leopard Frog (*R. sphenocephala*)

4. Study System:

- The H-02 wetland complex is a surface flow constructed wetland located on the Savannah River Site, near Aiken SC.
- The complex was constructed in 2006-07 to alleviate water quality exceedances (Cu and Zn) to a nearby stream due to process and storm water discharge from several industrial facilities in H-Area.
- Water in the retention pond (see fig 1) has the highest levels of Cu, Zn, and pH, and after a residence time of several days water exiting the wetland cells has lower levels of these variables.
- Constructed wetlands can provide new habitat for local wildlife, but if poor water quality limits recruitment, these wetlands could become "population sinks."
- We are monitoring amphibian colonization of the wetlands, and evaluating the effects of elevated metal levels on amphibian success in both lab and field studies.

Figure 1. Sampling and experimental locations in the H-02 wetland complex. Effluent from industrial facilities and surface water runoff enters the retention pond near L1 and L2—the retention pond exhibits high concentrations of Cu and Zn. Water is piped from the retention pond to a splitter box on the east end of wetland cell 1, and flow is divided between the two cells. Water exits the system below L5 and L6, after a residence time of approximately 2 d. Amphibian sampling and experimental manipulations are conducted at locations L1 thru L6, which corresponds to a water chemistry gradient of decreasing metal concentration. Incoming adult amphibians and exiting juveniles are sampled at the drift fences on the north side.

5. Methods:

Laboratory trials

- Control water chemistry variables by using a standard mix of synthetic dilution water for toxicity tests using freshwater organisms
- Rear eggs in containers at five (from 0 to 150 ppb) Cu concentrations
- Rear larvae in 1-L containers at four to five Cu concentrations
- Record data on survivorship, larval period, body size
- Analyze tadpoles for metal concentrations
- Collect tissue for genetics

Fig. 2. A1: Leopard frog eggs, A2: Bufo eggs, A3: Gastrophryne eggs, B: larvalTx containers, C: Bufo tadpole, D: Bufo near metamorphosis, E: recent metamorph and F & G: leopard frog metamorph

6. Results:

Leopard frogs (*Rana sphenocephala*)

- Significant effect of
  - Cu treatment on egg survivorship
  - female (mother) on egg and larval survivorship
  - Cu treatment on mass of metamorphs
- Can survive to metamorphosis in 100 ppb Cu

Southern toads (*Bufo terrestris*)

- Egg survivorship significantly affected by:
  - Cu treatment
  - Source of female (reference wetland or H02 wetlands)
  - Female (nested within source)
- Larval trial in progress: to date there has been 100% mortality above 15 ppb Cu

Eastern narrowmouth toads (*Gastrophryne carolinensis*)

- Egg survivorship significantly affected by:
  - Cu treatment
- Larval study underway

Table 1. Statistical results from ANOVAs with percent survivorship as the dependent variable

Species: egg or larval	Source	DF	F-value	P-value
R. sphenocephala: eggs (trial 1)	Cu treatment	4	73.30	<0.0001
	Female	3	2.04	0.1162
R. sphenocephala: eggs (trial 2)	Cu treatment	4	41.83	<0.0001
	Female	2	17.67	<0.0001
R. sphenocephala: larvae	Cu treatment	3	2.96	0.09
	Female	3	9.66	0.0036
B. terrestris: eggs (trial 1)	Cu treatment	4	82.58	<0.0001
	Source of female	1	107.72	<0.0001
	Female (source)	6	4.91	0.0002
B. terrestris: eggs (trial 2)	Cu treatment	5	7.65	<0.0001
	Female	3	15.11	<0.0001
G. carolinensis: eggs	Cu treatment	7	335.82	<0.0001
	Female	3	0.92	0.435

R. sphenocephala *during metamorphosis. Metals were determined in homogenized, freeze-dried biological tissues by using microwave digestion of samples in trace-metal-grade nitric acid followed by analysis using inductively coupled plasma mass spectrometry (ICP-MS).*

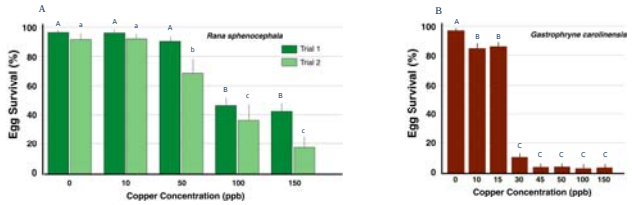

Figure 3. Results of egg survivorship trials for A) Rana sphenocephala, B) Gastroprhyne carolinensis, and C) Bufo terrestris. Treatments with significantly different means (by Tukey Studentized Range Tests) are indicated by different letters above the bars. Capital and small letters are used to differentiate separate trials. D) demonstrates the effect of female (dam) on egg survivorship in Bufo and E) illustrates species-specific differences in Cu sensitivity.

7. Conclusions:

- All three species exhibit Cu sensitivity
- Degree of sensitivity is species specific
- For two species (*R. sphenocephala* & *B. terrestris*) egg survival is strongly affected by the female (dam) and there is a strong female X treatment interaction (data not shown)
- The wetland source of the female matters: Eggs from female *Bufo* from Cu contaminated H-02 wetlands do worse in Cu treatments than eggs from reference site females
- Survivorship through the egg stage does NOT predict survivorship to metamorphosis

8. Genetics Research: In Progress

- Develop microsatellites via enrichment & 454 sequencing for all 3 species
  - Complete for leopard frogs
- Examine allele frequencies in parents and 24-48 larvae not used for experiment to insure Mendelian inheritance and establish starting "genepool"
- Compare allele and genotype frequencies among: starting genepool, survivors of egg trial, and survivors of larval trials (metamorphs)
- We collected livers from metamorph leopard frogs—now we will extract RNA and compare transcriptomes among treatment groups using massively-parallel sequencing (Illumina platform)

9. Genetics Research: Future Plans

- Take advantage of PacBio SMRT system coming to SC
- Conduct whole genome association mapping studies
- Explore genetic basis for variation in resistance among females
- Explore genetic basis for variation life history traits including:
  - Egg viability
  - Larval viability
  - Time to metamorphosis
  - Size at metamorphosis

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