

## EFFECTS OF PARENTAL AND EARLY LIFE EXPOSURE TO METALS ON GENOME METHYLATION IN TWO ANURAN SPECIES

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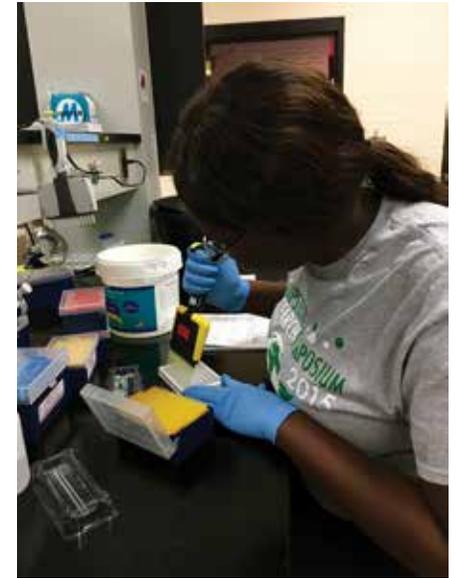
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Amphibians respond to metal contaminants in a variety of ways. Species and populations differ in their tolerance to elevated levels of metals in the environment, yet little is known about the mechanisms driving this variation in tolerance. DNA methylation is an epigenetic mechanism that regulates gene expression and can be altered by metal exposure. We designed our study to quantify how 1) early life exposure and 2) parental exposure to heavy metals affect genome methylation of two amphibians, southern toad (*Anaxyrus terrestris*) and eastern narrowmouth toad (*Gastrophryne carolinensis*). We collected adults from two metal-contaminated and two non-contaminated wetlands on the Savannah River Site, bred adults in the lab, and assigned offspring to one of three copper treatments in a factorial design. Embryos were kept in their treatments until after hatching (GS25), when we collected them for DNA extraction. We quantified the relative percent genome methylation in the DNA samples using a 5-mC DNA ELISA kit. We found that relative percent DNA methylation was altered by both Cu expo-

sure and population metal exposure history in both species. Southern toad larvae exhibited reduced methylation when exposed to copper early in development, and offspring from the contaminated site showed lower levels of methylation independent of Cu treatment. Furthermore, Cu exposure appeared to have a weaker effect on methylation levels in offspring from the contaminated site relative to those from the reference site. Narrowmouth toad larvae showed decreasing mean levels of methylation with increasing Cu exposure in offspring from the reference site and increasing mean levels methylation with increased Cu exposure in offspring from the contaminated site. These data suggest that both early life and parental metal exposure could be affecting methylation levels in these species and that an epigenetic mechanism could explain some of the differences in metal tolerance between species and population.



*Nia in the lab.*