

Plasma vitellogenin and hormone levels in common snapping turtles (*Chelydra serpentina*) from concentrated animal feeding operation (CAFO) ponds versus a reference site.

Sara Rogers¹, Jennifer L. Meyer¹, Linda Lee², Dan Villeneuve³, Gerald Ankley³,
and Maria S. Sepulveda¹

¹Department of Forestry and Natural Resources, ²Department of Agronomy,
Purdue University, West Lafayette, IN, and

³U.S. Environmental Protection Agency, Mid-Continent Ecology Division, Duluth,
MN

Abstract

Runoff from land treated with animal manure may contaminate adjacent aquatic ecosystems and negatively impact organisms living in these environments. Of notable concern, influx of estrogens can result in endocrine disruption and affect reproduction in aquatic vertebrates. Vitellogenin (VTG) is a phosphoglycoprotein synthesized by the liver of mature female non-mammalian vertebrates following estrogen stimulus. VTG production is typically lacking in males, however its synthesis can be induced by exposure to exogenous estrogen and estrogen mimics. The objective of this study was to develop a method to compare plasma VTG concentrations in two populations of the common snapping turtle (*Chelydra serpentina*), one proximal to a Concentrated Animal Feeding Operation (CAFO) and another from an uncontaminated reference site. We also evaluated changes in sex hormonal levels between these populations. Turtles were bled during the reproductive season (May-June) in 2007-2009. Plasma proteins were separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and VTG was assayed with a phosphoprotein stain (Pro-Q Diamond). The most prominent band in SDS-PAGE gels was isolated, trypsin-digested, and the derived peptides were sequenced using MALDI-TOF/MS. A 13 amino acid peptide sequence was obtained that was homologous to a portion of VTG from the marine turtle, *Mauremys reevesii*, relative to the amino acid positions 73-85, that indicated the phosphoprotein-stained band was indeed VTG. VTG was detected in plasma samples from all females but absent

from male turtles from both sampling sites. Comparison of the relative abundance of VTG plasma concentration using SDS-PAGE showed that the average VTG concentrations were similar between the two female populations, but that relative VTG concentrations were more variable in the population from the CAFO site than the population from the reference site. Hormone concentrations were measured using radioimmunoassay in each of the plasma samples and will also be presented. Our method for VTG peptide detection will be a useful tool to analyze the potential impact of estrogen contamination on turtle vitellogenesis in samples collected from a wider range of aquatic environments in forthcoming research.