

Report for 2005CT76B: Gonadal Deformities in Connecticut Amphibians

Publications

- There are no reported publications resulting from this project.

Report Follows

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Gonadal Deformities in Connecticut Amphibians (Project # 2005CT76B) PI: David Skelly, Yale University

Problem and Research Objectives: Freshwater wetlands are the recipients of an enormous diversity of compounds created or modified by humans. Fates and effects of most are poorly known and difficult to forecast. The practical impossibility of screening all contaminants for multiple potential impacts means that scientists must rely on a variety of means to identify threats to environmental health.

The use of wildlife sentinels as indicators of environmental condition is rapidly gaining currency. Effectiveness of this approach relies on careful selection of species that are likely to be exposed to potential contaminants and that are readily sampled.

Recently, amphibians resident in freshwater wetlands have been identified as sentinel candidates. A number of studies show that amphibian development can be impacted by chemical contaminants at relevant doses or exposures.

These laboratory results have been complemented by a recent field study (Hayes et al. 2002) showing that natural populations of amphibians in the Midwestern United States exhibit rates of gonadal abnormalities of up to 92%. The presence of vertebrate populations with high rates of developmental abnormalities including hermaphroditism has generated widespread attention. These findings are particularly alarming as the amphibian populations in question were often living in freshwater environments that form public drinking water supplies. These patterns generated enough concern that the U.S. Environmental Protection Agency convened a Science Advisory Panel in 2003 to consider their implications (USEPA 2003). The EPA noted that it was critical for more field studies to be conducted to determine the scope of the problem.

The status of populations in the northeastern United States remains unknown.

Connecticut is a particularly appropriate location for further field study. The state has a diverse set of landscapes including extensive tracts of undeveloped forest, intensive agriculture, residential areas and large urbanized zones. This variation in land cover within a relatively small area is ideal for discovering whether developmental abnormalities are present and whether land use is associated with these patterns.

Project Objectives:

1. Determine whether gonadal abnormalities are present in amphibians in Connecticut.
2. Characterize observed deformities.
3. Estimate the association between deformity rate and landscape composition.

Methodology: We conducted a sampling survey of amphibians living in wetlands across Connecticut stratified across 4 land use types: urban, suburban, agricultural, and undeveloped. GIS coverage for the state of Connecticut was used to designate each wetland in one of the four categories based on the dominant cover type surrounding the wetland perimeter. Wetlands for examination were selected at random using the National Wetlands Inventory conducted by the U.S. Geological Survey. In identifying wetlands for study, we employed the

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Connecticut Land Use Land Cover Data Layer (LULC). The LULC is based on LANDSAT Thematic Mapper Satellite Imagery data with 23 categories of land use and land cover. We visited 138 wetlands. During initial visits we ground truthed land cover designations and assessed the presence of green frogs (*Rana clamitans*). In the end we found 23 wetlands which had ground truthed land cover designations that matched remotely sensed data and for which we could successfully recover adequate amphibian specimens.

There are few published studies documenting patterns of gonadal abnormalities in wild Ranid frog populations (Hayes et al. 2002). We based our analysis of gonadal abnormalities on recommendations produced as a part of a recent EPA Science Advisory Panel Report (U.S. EPA 2003). Frogs are being scored for the presence of secondary sexual characters (e.g., thumb pads, vocal sacs) and sexed by gonadal examination under a dissecting microscope. Gonads will be removed and fixed with Bouin's preservative. Fixed gonads are embedded in paraffin, sectioned at 5 μ , and stained with hematoxylin and eosin (Qin et al. 2003). Gonadal structure is examined under a compound microscope. A wide variety of amphibian gonadal abnormalities have been described ranging from abnormal pigmentation to hermaphroditism. Specimens will be scored for all varieties of abnormalities.

Principal research Findings: We collected a total of 369 green frogs from 23 different wetlands. To date we have completed dissections (removal of a gonad and preparation for sectioning) on 354 individuals; the remaining individuals were gravid females or were otherwise unable to be prepped for histological analysis. Of these, histological preparations have been completed on 72 individuals.

In response to our first research objective, we can report that gonadal abnormalities are present in wild Connecticut amphibians. Of the 72 individuals completed so far, we have found serious abnormalities in 8 individuals; a gonadal abnormality rate of 11%.

The deformities uncovered have all been of a single type: ovarian follicular tissue within a testis. This deformity has been reported in wild collected amphibians in the western U.S. as well as laboratory reared individuals exposed to pesticides.

Recognizing that we are only part way through our histological examinations, we can report preliminary evidence for an association between deformities and land cover type. All but one of the deformities reported so far have been recovered from suburban contexts. The remaining individual was recovered from an agriculture associated wetland. None have been found in urban or undeveloped land cover types.

References:

Hayes, T., K. Haston, M. Tsui, A. Hoang, C. Haeffele, & A. Vonk. 2002. Feminization of male frogs in the wild: waterborne herbicide threatens amphibians in parts of the United States. *Nature* 419:895-896.

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Qin, P., R. Cimildoro, D. M. Kochhar, K. J. Soprano, & D. R. Soprano. 2002. PBX, MEIS, and IGF-I are potential mediators of retinoic acid-induced proximodistal limb reduction effects. *Teratology* 66:224-234.