

An Integrated Immunological-GIS Approach for Bio-monitoring of Ecological Impacts of Swine Manure Pollutants in Streams

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Goals and Objectives:

- This research is predicated on the hypothesis that low levels of swine liquid manure slurry and anaerobic lagoon liquid released to open water cause changes in immunological response in fish and increase fish susceptibility to infection. The initial objectives, therefore, are 1) to evaluate this hypothesis through a series of laboratory immunological assays applied to the test organism, the fathead minnow (*Pimephales promelas*) and 2) to identify one or more assays for use as a bio-monitoring technique to detect ecological impact of manure pollution in nature. A subsequent task involves use of digital environmental databases that are maintained and managed by the USGS BRD Iowa Cooperative Fish and Wildlife Research Unit at Iowa State University. The objective is 3) to characterize a number of Iowa watersheds and stream systems according to their potential susceptibility to hog manure pollution and to use this information to design a water quality and fish sampling regime. Finally, water and fish collected at selected stream sites will be analyzed through a battery of chemical and immunological procedures with the objectives 4) to quantitatively measure ecological impact of manure pollution on the streams, and 5) to evaluate the utility of this approach as a biomonitoring tool for environmental protection agencies.
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Progress:

2003 – The fathead minnow colony was successfully established. We have developed and constructed the computer controlled flow through system, but water heating problems caused unexpected delays in the project timeline. We have developed the isolation technique for extracting leukocytes from fathead minnow kidneys. We finished the morphological and cytochemical characterization of prepared leukocytes. We have developed and optimized an assay for measuring production of reactive oxygen species in isolated neutrophils by the cytochrom C reduction method.

2004 - We have developed and optimized an assay for degranulation of primary granules. The assay is capable of detecting handling and crowding stress as well as differences in various stress-causing treatments (anesthesia procedures). We established baseline values for FHM neutrophil oxidative burst, myeloperoxidase content and degranulation. We have tested developed assays on several fish species (catfish, bluegill, largemouth bass). We have started production of GIS maps in order to determine possible manure/chemical loads within designated watersheds. We have tested electrofishing equipment and assayed fish samples with our developed techniques.

2005 - We expanded the battery of assays with NETs (neutrophil extracellular traps) release assay, and tested the existing battery of assays with different immunomodulators. We calibrated the assays to be used in fathead minnows and optimized assays for use on bluegills, largemouth bass, common carp and catfish. We have compiled a GIS map of surfaces likely exposed to manure loading and currently are preparing maps for use in flow path analysis. We continued sampling in order to optimize sample collection and laboratory procedures for rapid and efficient analysis of neutrophil function from field samples. USGS approved an extension of the project to September 2006.

2006 - Flow path analysis for the State of Iowa has been partially completed and the analysis is ongoing. Data on fish kills and Iowa Department of Natural Resources (DNR) fish community sampling was requested from the DNR and is included in the GIS analysis of the flow path. Laboratory testing of manure effects on neutrophil function is ongoing.

Future Plans:

We plan to complete laboratory trials of manure impact on neutrophil function in Spring 2007. We plan to complete GIS flow path analysis and determine hot and cold spots in impacted streams in Spring 2007. We plan to analyze long term fish kill and fish community sampling data and determine if there is correlation between sampling points and manure loading predicted by the GIS model. We plan to prepare and submit final report for the project in 2007.