Report as of FY2006 for 2002IA25G: "An Integrated Immunological-GIS Approach for Bio-monitoring of Ecological Impacts of Swine Manure Pollutants in Streams"

Publications

- Articles in Refereed Scientific Journals:
  - Palic, D., C.B. Andreasen, D.E. Frank, B.W. Menzel, and J.A. Roth. (2005). A rapid, direct assay to measure degranulation of primary granules in neutrophils from kidney of fathead minnow (Pimephales promelas Rafinesque, 1820). Fish and Shellfish Immunology 19(3), 217-227.
  - Palic, D., C.B. Andreasen, D.E. Frank, B.W. Menzel, and J.A. Roth. (2005). Gradient separation and cytochemical characterization of neutrophils from kidney of fathead minnow (Pimephales promelas Rafinesque, 1820). Fish and Shellfish Immunology 18(3), 263-267.
- Other Publications:
  - Palic, D., C.B. Andreasen, D.M. Herolt, B.W. Menzel, and J.A. Roth, 2006. Immunomodulatory Effects of B-glucan on Neutrophil Function in Fathead Minnows (Pimephales promelas Rafinesque, 1820). Developmental and Comparative Immunology (In press).
  - Palic, D., D.M. Herolt, C.B. Andreasen, B.W. Menzel, and J.A. Roth, 2006. Anesthetic Efficacy of Tricaine Methanesulphonate, Metomidate and Eugenol: Effects on Plasma Cortisol Levels and Neutrophol Function in Fathead Minnow (Pimephales promelas Rafinesque, 1820). Aquaculture (In press).

Report Follows

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

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Problem and Research Objectives

Thirty years after enactment of the Clean Water Act, 40% of our nation's rivers, lakes, and coastal waters are still considered unfit for fishing, swimming, drinking or aquatic life. The U.S. EPA identified agricultural operations as the primary cause of non-point source pollution in the nation's impaired rivers and lakes. At least 10% of the nation's impaired river miles are affected by pollution from livestock operations. In portions of the Midwest, confinement livestock operations are a particular problem in this regard. Cases of massive deaths of aquatic organisms, often referred to as fish kills, are an extreme manifestation of the ecological impact of fecal contamination. Typically, they result from high concentrations of toxic ammonia contained in the manure or from depletion of dissolved oxygen in the water caused by decomposition of the pollutant. Chronic effects of manure pollution are poorly known, because of the difficulty of measuring them and placing them in ecological context. Moreover, low-level delivery of fecal pollutants can portend larger catastrophic inputs, for example, when a gradually leaking storage lagoon eventually bursts or an erosive, manure-fertilized crop field receives heavy rainfall.

State and federal agencies engaged in reducing non-point source water pollution are interested in obtaining new technologies for identifying, measuring and anticipating pollution occurrence. Clearly, development of tools that could integrate biological and environmental information to produce site-specific predictive models for guiding pollution-prevention management practices is highly desirable. The proposed research would develop a novel tool that integrates molecular biological and ecological approaches to quantitatively evaluate environmental impacts of swine manure pollutants. Although the technique will be developed with specific reference to Midwestern waters, it will be more broadly applicable, both geographically and with reference to other forms of pollution that engender immune responses in animals. Thus, we believe that the technique has potential to be widely adopted by state and federal environmental management agencies.

The research conducted under this grant reflects the need for integrated, multidisciplinary approaches to deal with complex environmental issues. It combines physiological laboratory techniques, computer modeling of agricultural landscapes and non-point source pollution pathways, and field-based ecological analyses to create a new and integrated approach for evaluating impacts of livestock fecal contamination on Midwestern streams.

The research relates to two major priorities of the NIWR National Competitive Grants Program.

A) It complements work by the USGS related to non-point source pollution, contributing to development of integrated watershed decision support tools for assessing organics and microorganisms transport and fate, along with their effects on aquatic systems.
B) It promises development of a new water quality sensor technology that will be based on integrated methodologies and will provide results that are readily accessible through the Internet.

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, fish communities at selected stream sites will be analyzed through developed GIS tools to 4) 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.

Methodology

The fathead minnow is a native Iowa species, abundant and ubiquitous in small streams. Thus, it is a good choice as a representative of fish communities exposed to low level concentrations of swine manure pollutants released into Iowa waters. Moreover, it is commonly used as a standard bioassay organism in toxicological analyses, so there is substantial knowledge on its tolerance to a wide array of environmental physical conditions and pollutants. Additionally, colonies are easily established and maintained in the laboratory. Fathead minnows used for the experiment were raised in a controlled environment, without previous exposure to swine manure.

The immune response was determined by activity of phagocytic cells, through several forms of measurement. Evidence from limited research involving fish suggested that assays measuring respiratory burst and degranulation are useful for determining phagocytic function in fish, and the procedure also seems to hold promise as a bio-indicator for fish health.

Geographic Information Systems (GIS) technologies provide a tool to enter, store, manipulate and integrate geo-referenced data on, for example, landscape features, water

quality, and aquatic organisms. The project applied GIS technology and landscape modeling to calculate possible swine pollutant flow path patterns in Iowa watersheds having large hog confinements and in those where liquid manure fertilizer is applied on crop fields. Using this approach, we estimated temporal and spatial distribution of manure loads and concentrations that reach receiving waters. This provided the basis for determination of actual conditions of water quality and fish communities at stream sites selected to represent a range of calculated manure pollutant loadings. Ecological impact of the pollution was evaluated by the developed immunological assays performed on wild-caught fathead minnows and on collected data on water chemistry and fish community structure using avaialbel data from Iowa Department of Natural Reosurces. Statistical comparisons are ongoing between the calculated and measured evidence for the pollutant to determine the accuracy and reliability of the GIS-immunological approach in actual practice. As a further check on the procedure, Index of Biotic Integrity (IBI) values determined by Iowa Department of Natural resources in their stream monitorning program is used. The IBI is a commonly used bioindicator of stream environmental quality. It serves as a summary measure of biotic community response to pollution and other forms of habitat degradation. It is being used routinely for long-term environmental monitoring programs in Iowa and other Midwestern states. This design, therefore, allowed for comparisons between this established coarse-scale environmental indicator and the experimental fine-scale immunological indicator.

Principal Findings and Significance for Period from 03/2005 to 09/2006

The effects of acute and chronic stress on the fathead minnow neutrophil function *in vitro* and *in vivo* were determined. Fathead minnows exposed to acute and chronic stress conditions had significantly reduced degranulation, demonstrating that the degranulation assay can be used to measure both acute and chronic stress effects on neutrophil function in this species. This step demonstrated the capability of the assay to measure reduction in neutrophil function. Scientific background and baseline data for use of fathead minnow neutrophil function in future research and aquatic ecosystem evaluation was provided. Fathead minnows are shown to be a useful model to investigate neutrophil degranulation in fish exposed to different environmental conditions. Experiments characterizing effects of sublethal manure exposure on innate immune function in fathead minnows are underway and the data analysis is ongoing.

The spatial distribution of manure on fields has been investigated in the context of aquatic ecosystem health. The inter-relatedness of disposal facilities, management plans, field location, rate of manure application, and stream network were investigated using spatial analysis over different data sets. The GIS model was developed to identify stream sections that have high likelihood to encounter potential hazards for stream biota. Furthermore, analysis of watershed scale flow path was performed for the State of Iowa and evaluation of high and low risk sampling points and comparison with fish kills and population assessment data from Iowa Department of Natural Resources is ongoing.

Fig. 1. Spatial distribution of A: Animal waste control facilities with operating permits in Iowa (red), Manure Management Plans for Animal Feeding (green); and B: Fish kills (red), in The State of Iowa.

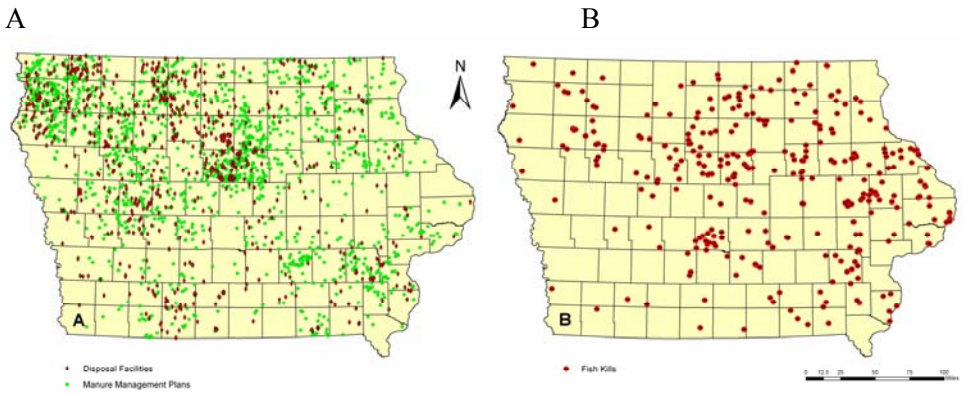


Fig. 2. Digitized fields used for manure disposal. Green: Agriculture Disposal facilities; Red: Manure Management Plans; Yellow: field boundaries. (Hamilton County)

Fig. 3. Predicted flow of manure after field application. Quantity of manure is expressed as intensity of color (dark red = more). Gray areas indicate potential critical points in streams (blue). (Hamilton County)

Figure 2. Figure 3.

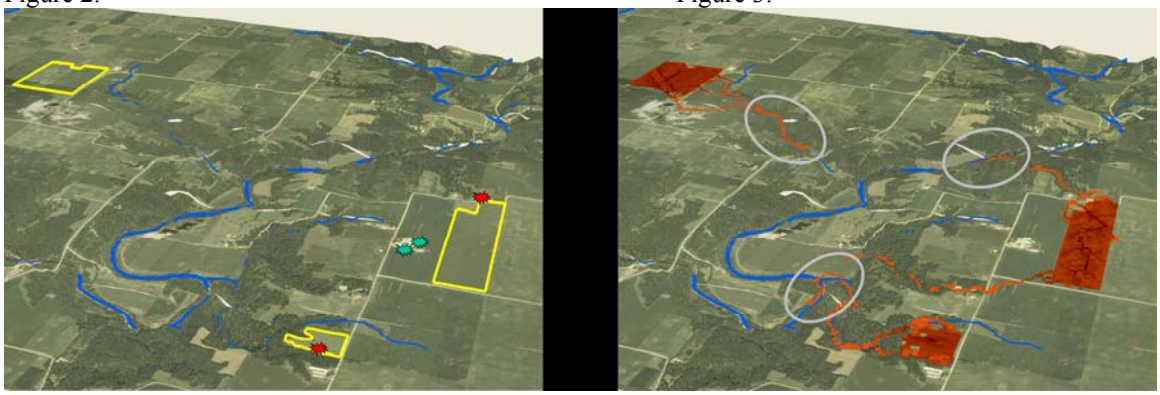


Fig. 4. Three possible scenarios for manure flow based on differences in land cover. Dark green: forest (A); Light green: grassland (B); Tan: row crops (C). Quantity of manure is expressed as the intensity of color (dark red = more). Black arrows indicate the distance of manure flow in streams (blue). (Hamilton County)

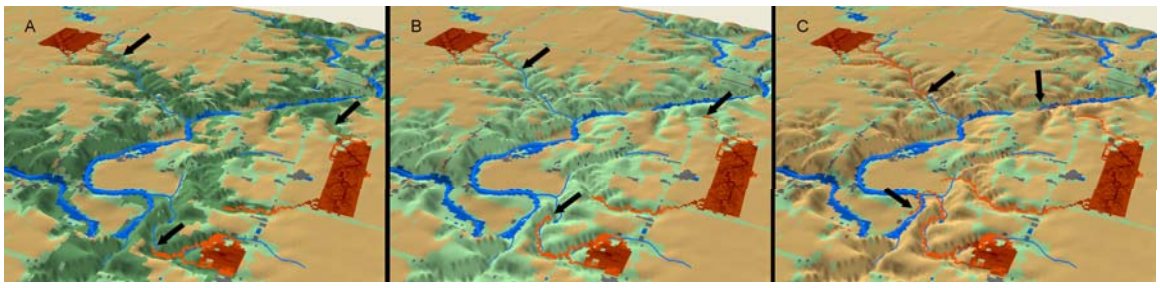
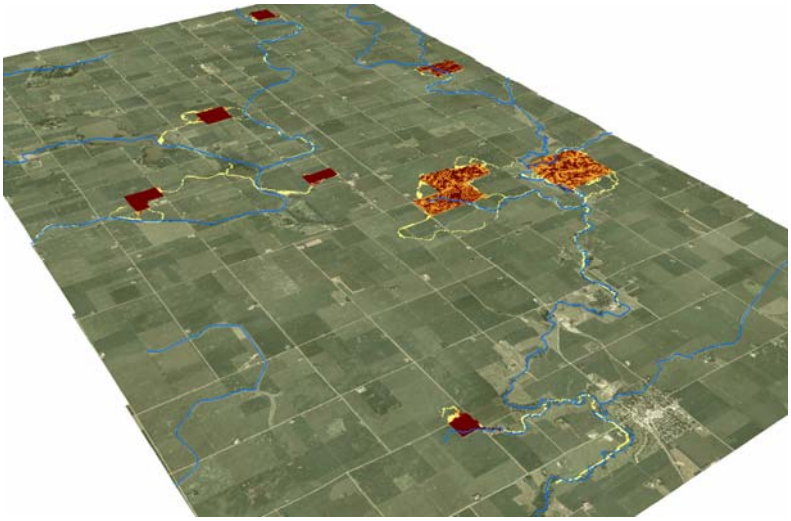


Fig. 5. Example of county (watershed) scale flow path anlysis with identification of critical points of potential manure inflow in public and private waterways. This flow path anaysis is used on state-wide scale to investigate potential correlation of manure exposure, fish kills, and fish community structure (IBI).



Using spatial distribution of AGDs, MMPs, and fish kills, the areas with potential environmental concern were identified in northeast and northwest Iowa (Fig 1.). Based on data accessibility, Hamilton County was selected for detailed study. Conversion of vector to raster datasets, calculations of manure flow path, and setting the threshold level were performed in ArcGIS for different field situation scenarios. The manure flow from three different fields was calculated and critical points in stream were identified (Figs 2-4.). The critical points were determined based on the presence of the manure in stream sections above the threshold level. The difference in land cover has a significant effect on the flow path length and quantity of manure reaching streams ($P<0.01$). The flow of manure through forested areas is the shortest and through row crop is the longest (Fig 4.). The anal;ysis was repeated for total of 12 counties, including Emmet county (Fig 5.).

Based on preliminary analysis of available datasets, we suggest that the TauDEM flow path analysis and the developed geospatial model have potential to be used as a tool to determine stream sections with increased probability of critical impairment due to manure contamination. A fully developed tool could assist in the selection of stream sampling points relevant to ecosystem health assessment and reduce the cost of field surveys. Changes in land cover can be modeled to help management decisions, improve spatial distribution of animal facilities, and predict possible outcomes of restoration efforts.

In summary, work performed from 03/2005 to 09/2006 clearly demonstrated that:

1. Neutrophil functional assays show potential for use in studying effects of immunomodulatory compounds, as well as effects of environmental stress on fish physiology, providing us with new tools to be used in the assessment of aquatic ecosystem health.

2. The TauDEM flow path analysis and the developed geospatial model have potential to be used as a tool to determine stream sections with increased probability of critical impairment due to manure contamination. A fully developed tool will assist in the selection of stream sampling points relevant to ecosystem health assessment and reduce the cost of field surveys.
3. Changes in land cover can be modeled to help management decisions, improve spatial distribution of animal facilities, and predict possible outcomes of restoration efforts.