Title
Climate change and restoration factors affecting fecal pathogen dynamics in wetland systems

Authors
Miller, Woutrina A.
Watson, Fred

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Climate change and restoration factors affecting fecal pathogen dynamics in wetland systems

Woutrina A. Miller
530-219-1369
wamiller@ucdavis.edu

Fred Watson
831-582-4452
Fred_Watson@csumb.edu

Project Hypothesis

This project has three main hypotheses:
1) Increased water temperatures, decreased salinity, and increased flow rate will enhance transport of protozoal oocysts through wetland systems.
2) Larger vegetation, increased vegetation density, and increased wetland length will enhance retention of protozoal oocysts in wetland systems.
3) Factors found to maximize protozoal retention in the laboratory-based models will also significantly affect protozoal transport in a natural and constructed wetland.

Project Goals and Objectives

Study Goal: Determine how factors and processes involved in 1) climate change and 2) wetland restoration affect fecal pathogen transport in wetland systems.
Objective 1: Evaluate and model how climate change variables such as water temperature, salinity, and flow affect transport of fecal protozoal pathogens and surrogates in controlled laboratory-based experiments that mimic coastal wetland conditions.
Objective 2: Evaluate and model how wetland restoration variables such as vegetation type, vegetation density, and wetland length affect transport of fecal protozoal pathogens and surrogates in controlled laboratory conditions that mimic coastal wetland conditions.
Objective 3: Compare the laboratory findings with field experiments by studying the transport and retention of protozoal oocysts and/or surrogates in a natural and constructed wetland.

Briefly describe project methodology

This project utilized two approaches to examine wetland factors associated with protozoal retention: laboratory experiments and field studies. The laboratory experiments involved vertical settling columns and re-circulating mesocosm tanks and the field studies occurred on the central coast of California. We examined protozoal transport at three separate locations in the field: a tidal wetland, an ephemeral wetland passing through a dairy, and a constructed research wetland.

The settling columns were 1 L in volume with side ports to take water samples at time-points of interest to determine oocysts settling rates. Factors hypothesized to affect oocyst settling, such as salinity, water temperature, sediment concentration, sediment particle size, and water type (nano-purified versus environmental water with natural sediment) were measured during each experiment. Samples were stained with direct fluorescent antibody (DFA) assays for visualization and enumeration of protozoa and particles. The re-circulating mesocosm tanks provided a more realistic natural environment to observe protozoal transport than the settling columns. These tanks were 3 m long and 0.5 m wide, and held 450 L of water when filled to a depth of 0.3 m. Protozoa (*Cryptosporidium parvum*, *Giardia lamblia*, and surrogate microspheres for *Toxoplasma gondii*) were pulse injected at the beginning of the simulated channel and samples were taken at specific time points post-injection. Experiments examined the effect of salinity, flow rate, and presence of vegetation on oocysts transport. Immunomagnetic separation (IMS) was performed on the samples to isolate the protozoa, and direct filtration was used to isolate the microspheres. Protozoa samples were DFA stained for visualization and enumeration.

The field studies allowed us to study protozoal transport dynamics in more natural conditions and to compare findings with those observed in laboratory experiments. The constructed research wetland was utilized to evaluate climate change and wetland restoration factors in natural systems using both ambient monitoring and release studies. The tidal wetland and the dairy wetland were evaluated using ambient monitoring.

The constructed wetland has two delineated sections, upper and lower. The upper portion is a sinuous channel with a length and width of 282 m and 6 m respectively, has a maximum depth of 0.40 m, and volume of 800 m3. The lower section is a pond feature with an average depth of 0.15 m and volume of 400 m3. Ambient monitoring occurred over 2 years and consisted of sampling both the upper and lower section for protozoa during dry and wet seasons. Release studies occurred in the upper section within a flume and consisted of continuously injecting surrogate microspheres at a controlled rate and monitoring concentration downstream.

The tidal wetland has a maximum channel depth and width of approximately 0.5 m and 150 m respectively and channel length of 4000 m depending on season and tide. The dairy wetland is a seasonal wetland that receives run-off from cattle pastures, and drains into the tidal wetland described above. We monitored for protozoa at varying distances along the channels of the tidal and dairy wetlands to determine the effect of channel length and water quality parameters on pathogen dynamics.

Describe progress and accomplishments toward meeting goals and objectives.
During the first three years of this project, as well as the 9-month no-cost extension, we have met all three of our objectives and accomplished all of the planned experiments. All planned laboratory experiments have been conducted for both the mesocosm tank and settling column platforms following preliminary experiments, which informed the development of methods for sample collection and sample processing optimization. Tracer studies and protozoal transport studies were conducted in the mesocosm tanks at different water velocities, salinity concentrations, and vegetation configurations. Ambient water quality monitoring at the wetland field study sites is also complete. Following tracer studies at the constructed research wetland, release studies using microsphere surrogates for Toxoplasma to compare their transport to the settling column and mesocosm tank experiments, has been completed. A manuscript detailing results of the field water quality monitoring has been submitted to a peer-reviewed journal for review and a manuscript detailing the settling column and mesocosm tank experiments is currently in preparation. Additionally, we have developed our pathogen fate and transport model to account for the effect advection, dispersion, and environmental factors (suspended solids, salinity, water temperature, and vegetation) have on protozoa in coastal California wetlands. We are currently implementing our model in a Bayesian statistical framework and drafting a manuscript to be submitted to a peer reviewed journal.

Objective 1. Evaluate and model how climate change variables such as water temperature, salinity, and flow rate affect transport of fecal protozoal pathogens and surrogates in controlled laboratory-based experiments that mimic coastal wetland conditions.

Objective 1 is complete. Laboratory-based settling column experiments to determine the settling velocity of protozoa in an environment assumed to have no advection and dispersion have been completed. These experiments investigated variables including water temperature, salinity, suspended sediment concentration, and particle size. The wetland mesocosm tank experiments have been completed to examine the effect of salinity and flow rates on the retention of protozoa with and without vegetation. Two flow rates (1cm/sec, 0.1 cm/sec) and two levels of salinity (0.1ppt, 30ppt) were used to parameterize what we have observed in natural coastal wetlands in central California. All experimentation and analysis is completed for this objective. A manuscript is in preparation to disseminate results to the scientific community.

Objective 2. Evaluate and model how wetland restoration variables such as vegetation type, vegetation density, and wetland length affect transport of fecal protozoal pathogens and surrogates in controlled laboratory conditions that mimic coastal wetland conditions.

Objective 2 is complete. Wetland mesocosm tank experiments have been completed to examine the effect of vegetation on the retention of protozoa. Transplanted California bulrush (*Schoenoplectus californicus*) and slough sedge (*Carex obnupta*) were both used to examine the effects differing vegetation species may have on fecal pathogen removal. Vegetation configuration was examined using California bulrush in two configurations (buffer strip of vegetation across the tank and channel where water flows between two stands of vegetation) to parameterize the effect of vegetation within a natural wetland system. Preliminary trends regarding the effect of wetland length have been evaluated through our sampling of the tank experiments and field wetland types. These trends were investigated further in the field studies, where we performed a comparative study within the constructed research wetland. The comparative study consisted of manipulating the density of vegetation in the channel to infer the effect of vegetation on protozoal removal. All experimentation and analysis is completed for this objective and manuscripts are in preparation to disseminate results to the scientific community.

Objective 3. Compare the laboratory findings with field experiments by studying the transport and retention of protozoal oocysts and/or surrogates in a natural and constructed wetland.

Objective 3 is complete. Monitoring of protozoa in the constructed wetland, dairy wetland, and a natural tidal wetland have all been completed. This monitoring is beneficial in that it informed us of the prevalence of the parasites of interest in these three wetlands. A manuscript detailing this field monitoring is currently in review. Monitoring has also provided information regarding general wetland characteristics for model parameters. The field release studies have also provided insight into protozoal transport not easily obtained through ambient monitoring and have increased our ability to estimate processes controlling the fate and transport of protozoa in coastal California wetlands. A manuscript describing these experiments is currently in development for submission to a peer reviewed journal.

PROJECT MODIFICATIONS:
In addition to our planned objectives and pre-identified factors affecting protozoal transport, we identified two supplementary components that we were able to address in our studies: sediment dynamics and protozoal hydrophobicity. During year 1 of this project, it was determined that concentration and particle size of suspended sediment may have a significant effect on the retention of protozoa in the water, and so we included sediment studies alongside the protozoal studies in both the mesocosm tanks and settling columns. Secondly, in discussions with our collaborative researchers, we realized that hydrophobicity of protozoa could lead to more parasites associating with sediment particles and thus settling out of the water column more efficiently. We are continuing to collaborate with other researchers in an effort to better understand the importance of hydrophobicity and what this may mean for protozoal transport dynamics in coastal watersheds.

Over the course of this project there have been minor modifications to the proposed timeline in order to focus on development of new tank methodology, to investigate the potential for sediment association with the pathogens of interest, and to redesign experiments dependent on infrastructure that was stolen. We sampled the constructed wetland site only two months in the first year instead of the originally proposed four sampling months, and thus our wetland sampling occurred over a two year time period and is now complete. We were additionally delayed in the hydrological assessment of the constructed wetland due to unexpected setbacks. These setbacks included theft of solar panels, on two separate occasions and despite being secured, for the wetland pump and other equipment, which was covered by the UC Davis insurance policy. While the use of solar power for this field site would be beneficial, the security of the site and setbacks that have been incurred suggest that running the pump off the regular power grid is a better choice for this project. This power transition was completed in September 2010, which delayed the start of the field release studies, but they have now been completed. This delay impacted the data analysis/synthesis phase of the project into the no-cost extension period.

PROJECT OUTCOMES:
This project has produced important findings regarding the transport of Cryptosporidium and Giardia in California coastal wetlands, as well as with regard to specific factors evaluated within experimental settling column and mesocosm tank experiments. Fecal protozoa and fecal indicator bacteria were present in California coastal wetlands and the concentrations of these parasites were reduced at downstream sample locations within a wetland. The experimental studies were conducted to determine specific factors leading to this potential reduction and it was found that vegetation and likely suspended solids play a key role in reducing the number of parasites from the water with the effects of water temperature and salinity less certain. Release studies at the research wetland confirmed that the presence of vegetation in a wetland channel facilitates the reduction of protozoa from upstream sources. Drawing from our results we conclude that wetlands, when adequately vegetated, are a practical consideration for addressing protozoal pathogen pollution in Monterey Bay watersheds, and that an increase in vegetated wetland area would likely correspond to an increase in water quality improvement for the region.

We have also developed new experimental methods, data sets, and models to examine protozoal fate and transport in wetland systems. The use of re-circulating mesocosm tanks represents a novel semi-controlled method developed under this study and has the potential to be used for other projects in the future. Additionally, we have produced new datasets relating to climate change scenarios and wetland restoration effects on pathogen retention, and we have characterized properties of the tidal wetland and the constructed wetland that may benefit stakeholder groups. We have also developed a simulation model capable of functioning at the settling column, mesocosm tank, and wetland scale that is capable of evaluating climate change and wetland restoration factors with regard to pathogen removal. By approaching these research questions from laboratory, field, and modeling aspects, we have taken a comprehensive approach to evaluating and identifying factors for future research as well as to informing science-based management decisions by resource managers that will further and improve water quality.

IMPACTS OF PROJECT:
We foresee several significant impacts of this project. First, a valuable set of tools and knowledge have been collected and will be disseminated for regulating bodies, stakeholders, and the scientific community who require scientifically justified evidence evaluating methods to reduce the impacts of protozoal pollution on coastal ecosystems. Additionally, many of these approaches and findings can be applied to restoration of wetlands that will benefit public health and specifically reduce exposures of waterborne fecal pathogens for humans, animals, and wildlife. Third, the study findings and associated model will be of use to resource agencies charged with watershed management, species conservation, and coastal ecosystem restoration.

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS:
We expect this project to benefit water quality stakeholder groups, including the State Water Quality Control Board, Southern Sea Otter Alliance, and local watershed protection or restoration groups.

ECONOMIC BENEFITS generated by discovery
This project has potential economic benefits through the protection and restoration of natural wetlands. Wetlands provide a number of ecosystem services, including filtration of water contaminants including fecal pathogens, and natural habitat for a variety of wildlife. Our research findings can improve the cost-effectiveness and efficiency of wetland restoration efforts by providing a larger foundation

of oocyst fate and transport knowledge in wetland environments to inform and prioritize mitigation strategies, and by developing a simulation model capable of aiding in the design phase of wetland restoration and construction in the future.

Issue-based forecast capabilities

This project has a number of forecast capabilities, which adds to its interest and importance. Primarily, this project has the potential to inform stakeholders on the effects climate change may have on retention of pathogens in wetlands in central California. Additionally, this project examines pathogen pollution in wetlands on the central coast of California and will provide information on ways to reduce pathogen loads using wetlands. This project may also offer suggestions for wetland restoration, and preservation of the remaining wetlands. Ultimately, the data from this project has the potential to impact public health and water quality.

Tools, technologies and information services developed

The website www.pathogenpollution.org is hosted by the University of California, Davis and was created to engage the public and to provide resources of interest to school groups as well as resource management agencies. Research findings derived from this Sea Grant project will be disseminated using this website to post links to peer-reviewed publications, lay summaries, and educational materials for use in schools as a way to foster dialogue on pathogen pollution.

Publications

<u>Peer-reviewed journal articles or book chapters</u>

Hogan JN, Daniels ME, Watson FG, Conrad PA, Oates, SC, Miller MA, Hardin D, Myrne BA, Dominik C, Melli A, Jessup DA, Miller WA. Epidemiology of Cryptosporidium, Giardia, and fecal indicator bacteria in coastal California wetland ecosystems. *Water Research*. In review.

Hogan JN, et. al. Vegetative removal of *Cryptosporidium parvum*, *Giardia lamblia*, and Toxoplasma surrogates in water. In preparation—to be submitted.

Daniels MD, et. al. Estimating environmental conditions affecting protozoa removal in wetland systems using a multi-scale model-based approach. In preparation.

<u>Other</u>

Hogan JN. Epidemiology of Fecal Protozoa at the Human, Animal, and Environmental Interface. Dissertation for University of California, Davis, Ph.D. in Epidemiology. Expected completion date June 2012.

Daniels ME. Estimating environmental conditions affecting protozoa removal in wetland systems using a multi-scale model-based approach. California State University, Monterey Bay. Expected completion date Spring 2012.

WORKSHOPS AND PRESENTATIONS:

Hogan J, Daniels M, Watson F, Conrad P, Miller, W. Waterborne Protozoal Removal by Coastal California Wetlands. Oral presentation given at American Society of Parasitology Annual Meeting: Anchorage, AK, June 1-4, 2011.

Daniels M, Watson F, Miller WA, Hogan J. Estimating protozoal fate and transport in a wetland system using a model-based approach. Oral presentation at the Headwaters to Ocean Conference, San Diego, CA, May 2011.

Daniels M, Watson F, Miller WA, Hogan J. Estimating protozoal fate and transport in a wetland system using a model-based approach. Poster presented at the Monterey Bay Sanctuary Currents Symposium, Monterey, CA, April 2011.

Hogan J, Daniels M, Watson F, Conrad P, Miller, W. Waterborne Protozoal Removal by Coastal California Wetlands. Poster presented at EPA National Beach Conference: Miami, FL, March 14-17, 2011.

Daniels M, Watson F, Miller WA, Hogan J. Estimating protozoal fate and transport in a wetland system using a model-based approach. Poster presented at the EPA National Beach Conference, Miami, FL, March 2011.

Hogan J, Daniels M, Watson F, Conrad P, Miller, W. Waterborne Protozoal Removal by California Coastal Wetlands: The Role of Vegetation. Oral presentation given at Northern California Branch of American Society of Parasitology: Tiburon, CA, March 12, 2011.

Daniels M, Watson F, Miller WA, Hogan J. Estimating protozoal fate and transport in a wetland system using a model-based approach. Poster presented at the California and World Oceans Conference, San Francisco, CA, September 2010.

Hogan J, Daniels M, Watson F, Conrad P, Miller, W. Waterborne Protozoal Removal by California Coastal Wetlands: Toxoplasma Surrogate Microspheres. Oral presentation given at Northern California Branch of American Society of Parasitology: Point Reyes, CA, March 13-14, 2010.

Hogan J, Daniels M, Watson F, Conrad P, Miller, W. Waterborne Protozoal Removal by California Coastal Wetlands: An Overview. Oral presentation given at Northern California Branch of American Society of Parasitology: Point Reyes, CA, March 8-9, 2008.

COOPERATING ORGANIZATIONS:
State
Marine Wildlife Veterinary Care & Research Center
California Department of Fish and Game

Nongovernment
Applied Marine Sciences and Central Coast Long-term Environmental Assessment Network

Academic
University of California, Davis
California State University, Monterey Bay

INTERNATIONAL IMPLICATIONS:
Wetland processes and models in California will inform water quality management and wetlands restoration in the US as well as in other countries. A new research proposal was submitted in 2012 to the National Science Foundation for similar wetlands research to be conducted in Tanzania, led by Dr. Njau at the Nelson Mandela African Institute of Science and Technology, in partnership with Dr. Miller at UC Davis.

FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:
Volunteer Count : 2

Graduate Student Info
Jennifer N. Hogan
UC Davis
Department of Vet Med: PMI
jnhogan@ucdavis.edu
650-4308-741
Degree Program: PhD, Epidemiology
Thesis Title: Epidemiology of Fecal Protozoa at the Human, Animal, and Environmental Interface
Supported by Sea Grant: Yes
Start Date: 2/1/2008 End Date: 1/31/2011

Miles E. Daniels
CSU Monterey Bay
Department: Science and Environmental Policy
831-392-6913
mdaniels@csumb.edu
Degree Program: M.S.
Thesis Title: Modeling fate and transport of fecal pathogens in wetland systems on the central coast of California
Supported by Sea Grant: Yes
Start Date: 2/1/2008 End Date : 1/31/2011

KEYWORDS:
wetlands, protozoa, pathogen transport, Cryptosporidium, Giardia, transport modeling, water quality, Cryptosporidium, Giardia, wetland, protozoa, coastal