Role of Bacteria as Indicator Organisms for Watershed Assessment and in Determining Fish Pathogen Relationships with Fauna of Abernathy Creek

ProjectID: 30013

Response to ISRP
Preliminary Report

ProjectID: 30013
Role of Bacteria as Indicator Organisms for Watershed Assessment and in Determining Fish Pathogen Relationships with Fauna of Abernathy Creek
Sponsor: USFWS
Province: Columbia Estuary
PI: Richard Glenn

Comment 1: ***The test proposed will not likely provide the intended relationship between watershed health and microbial community structure. Further detail needs to be provided on this subject.***

I am writing in response to the ISRP concerns about my proposed project (Project 30013) to examine the relationship between watershed health and the microbial community structure. I plan to monitor and collect environmental data on: pH; water temperature; total hardness; dissolved oxygen; ammonia; nitrate; nitrite; turbidity; carbon dioxide; chloride; alkalinity; and phosphate levels. In addition to the environment data, land practices will be determined by the use of survey maps to show where logging, housing, and land use are occurring in the watershed.

After a preliminary consultation with a biostatistician, I will probably use logistic regression or principle components analysis (PCA) to examine the relationships between the microbial community and the environmental factors. The logistic regression will allow the analysis of my categorical response variables, the presence or absence of a bacterial species, given the measurements of the environmental and land use independent variables. The PCA will allow me to make a univariate "composite" of the multivariate independent variables for comparison to microbial community structure. Specifically, the PCA will allow the data of the presence or absence of other microbial species to be used as variables in determining inter-bacterial relations, bacterial vs aquatic life relationships, and bacterial vs environment. By using these statistical models, determination of a bacterial species as indicators of watershed health should hypothetically be possible. These types of multivariate analyses will require working closely with a statistician to ensure proper sampling design, data analysis, and interpretation.

The survey map analysis and environmental data collection are not expensive and require minimal time. The majority of the work and expense will be the bacterial assessment of the microbial community. I have added collection of environmental data in the Monitoring and Evaluation Phase of the project and on the Administering and Budgeting part of the proposal. These changes will add a small additional cost of approximately $2350 the first year and $250 for each of the next two years. These monies would allow me to purchase 10 temperature loggers, a portable turbidity meter, and upgrade the Center's water quality test kits.

There were concerns during my oral presentation about differences in the microbial structure at each sample site and which bacterial species would be used as indicator species. The proposed project does not intend to relate the entire microbial species community structure to watershed health. This is technologically impossible at this time due to limiting factors such as non-culturable bacteria, the inability to re-isolate others, and the complexity of the bacterial community. This project proposes to screen for organisms that have been shown in previous research to be widespread and to be correlated to habitat quality. The following bacteria will be organisms of interest:

Species	***Commonly found in***	***Relationship to Environment***	***Reference***
Acinetobacter calcoaceticus	soil and water	thrives in polluted areas	Lemke et. al., 1997; Lemke and Leff, 1999

Aeromonas caviae	common in streams	possible inverse relation to allochthonus or benthic imputs	Janakiraman and Leff, 1999
Aeromonas salmonicida	fish, lake and streams (1)	fish pathogen, otherwise unknown relation to environment	Morgan et. al., 1992; Pickup and Rhodes, 1997
Aeromonas spp.	widespread in nutrient rich waters and sewage	increase in concentration may indicate fecal contamination in surface waters	Brion et. al., 2000
Bacillus thuringiensis	insects, soils, water	insect pathogen, indicator of insecticide use	Janakiraman and Leff, 1999
Beijerinckia spp.	soils, in acid environments, water	acid tolerant, possible indicator of acidity	Holt, et. al., 1994; Glenn, raw data
Burkholderia (Pseudomonas) cepacia	common in streams and soils	possible relation to allochthonus or benthic imputs, does not thrive in polluted waters	Leff et. al., 1995; Lemke et. al., 1997; Leff et. al., 1998; Janakiraman and Leff, 1999; Lemke and Leff, 1999; McArthur et. al., 1992
Flavobacter psycrophila	salmonids, freshwater	fish pathogen	
Flavobacterium spp.	soil and water	common bacteria	Holt et. al., 1994; Leff et. al., 1994
Lampropedia spp.	organically rich environments, freshwater	common bacteria, found in Abernathy Creek	Holt et. al., 1994; Glenn, raw data
Proteus vulgaris	human and animal commensal parasite, water	indicator of fecal material	Holt et. al., 1994; Glenn, raw data
Pseudomonas aeruginosa	soil and water	denitrifier, possible indicator of “stored” rich nutrient water.	Ellis and Yu, 1995; Brion et. al., 2000

Pseudomonas pickettii	natural materials, freshwater, and soil	common bacteria	McArthur et. al., 1992; McArthur and Tuckfield, 2000
Pseudomonas putida	soil, water, mineral enrichments	found to degrade recalcitrant carbon compounds, otherwise has an unknown relation	Lemke et. al., 1997; Lemke and Leff, 1999
Vibrio spp.	require saline environments	pathogenic, saline environments	Brion et. al., 2000
Xanthomonas spp.	associated with plants	common bacteria	Holt et. al., 1994

1. *Aeromonas salmonicida* is culturable in nutrient rich waters, but has been found to enter into the non-culturable but viable (NCBV) state at reduced nutrient levels.

Comment 2: ***It is unclear whether the PI is qualified to do this work (BS in Chemistry, limited research experience); it's unlikely that a GS5 Technician, however capable, would be able to fulfill the responsibilities of a PI.***

An additional concern expressed by the ISRP is my role as the principle investigator. Since submitting this proposal I have been promoted to the position of Microbiologist, GS-5/7/9 at the U.S. Fish & Wildlife Service, Abernathy Fish Technology Center. My new position has the responsibilities of a trainee in a professional job series. In this position my duties and responsibilities will increase until my skills and work experience are equivalent to those of a Master's degree recipient. If funded, I will explore the possibility of using this research to obtain a Master's degree. As such, my work under this proposed project will be under the complete oversight of Dr. Peter Taylor, head of the Applied Research Program in Pathology at the U.S. Fish & Wildlife Service, Abernathy Fish Technology Center (see attached curriculum vitae). Dr. Taylor has 35 years of research experience in the fields of microbiology and pathology. In addition to his numerous publications, he is currently serving as the technical expert on a committee undertaking the national standardization of bacteriological protocols for the Service's Fish Health Centers. He also serves on two professional certification boards (fishery scientists and fish pathologists) for the American Fisheries Society, the primary organization for fishery professionals in North America.

References

Brion, G. M., H. H. Mao, and S. Lingireddy. 2000. New approach to use of total coliform test for watershed management. Water Science and Technology 42(1-2): 65-69

Ellis, J. Bryan and Wang Yu. 1995. Bacteriology of Urban Runoff: The Combined Sewer as a Bacterial Reactor and Generator. Water Science and Technology 31(7): 303-310

Holt, John G., Noel R. Kreig, Peter H. A. Sneath, James T. Staley, and Stanley T. Williams. 1994. Bergey's Manual of Determinative Bacteriology, 9th ed. Williams and Wilkins, Baltimore, Md.

Janakiraman, Anuradha and Laura G. Leff. 1999. Comparison of survival of different species of bacteria in freshwater microcosms. Journal of Freshwater Ecology 14(2): 233-240

Leff, Laura G., J. Vaun McArthur, Judy L. Meyer, Lawrence J. Shimkets. 1994. Effect of macroinvertebrates on detachment of bacteria from biofilms in stream microcosms. Journal of The North American Benthological Society 13(1): 74-79

Leff, L.G.; R.M. Kernan, J.V. McArthur, and L.J. Shimkets. 1995. Identification of aquatic *Burkholderia (Pseudomonas) cepacia* by hybridization with species-specific rRNA gene probes. Applied and Environmental Microbiology 61(): 1634-1636

Leff, Laura G., Adam A. Leff and Michael J. Lemke. 1998. Seasonal changes in planktonic bacterial assemblages of two Ohio streams. Freshwater Biology 39: 129-134

Lemke, M.J.; B.J. Brown; L.G. Leff. 1997. The response of three bacterial populations to pollution in a stream. Microbial Ecology 34:224-231

Lemke, M. J. and L. G. Leff. 1999. Bacterial Populations in an Anthropogenically Disturbed Stream: Comparison of Different Streams. Microbial Ecology 38: 234-243

McArthur, J. Vaun; Laura G. Leff; and Michael H. Smith. 1992. Genetic diversity of bacteria along a stream continuum. Journal of the North American Benthological Society 11(3): 269-277

McArthur, J. Vaun and R. Cary Tuckfield . 2000. Spatial Patterns in Antibiotic Resistance among Stream Bacteria: Effects of Industrial Pollution. Applied and Environmental Microbiology 66(9): 3722-3726

Morgan, J. A. W., K. J. Clarke, G. Rhodes, and R. W. Pickup. 1992. Non-culturable *Aeromonas salmonicida* in lake water. Microbial Releases 1:71-78

Pickup, R.W. and G. Rhodes. 1997. Detection of specific bacteria in water: implications of survival strateg. The Microbiological Quality of Water. Freshwater Biological Association, Ambleside. 28-34pp.

Attachment 1

PETER W. TAYLOR

POSITION: Head Pathologist
Pathology Applied Research Program
U. S. Fish & Wildlife Service
Abernathy Fish Technology Center
1440 Abernathy Creek Road
Longview, WA 98632
360-425-6072, ext. 228 voice
360-636-1855 FAX
pete_taylor@fws.gov

EDUCATION:

PhD. Fish Pathology, Auburn University, AL, 1977
M.S. Fish Diseases, Auburn University, AL, 1975
B.S. Fisheries Science, New Mexico State University, NM, 1974

EXPERTISE:

Fish pathology and microbiology with applications to fish health, management, conservation, and artificial propagation; use of innovative polymerase chain reaction techniques to identify and quantify fish pathogens. Conduct research on salmonid diseases affecting fish production in the Pacific Region (ID, OR, WA, CA, and NV) of the U. S. Fish & Wildlife Service.

PROFESSIONAL EXPERIENCE:

1996 - present: Head Pathologist, U.S. Fish & Wildlife Service, Abernathy Fish Technology Center, Longview, WA 98632.
1992 - 1995: Section Head, Pathology and Water Quality Section, National Biological Survey, Southeastern Fish Culture Laboratory, Marion, AL 36756.
1986 - 1992: Areas Fisheries Specialist, Cooperative Extension Service and the College of Veterinary Medicine, Mississippi State University, Belzoni, MS 39038.
1982 - 1984: Laboratory Director/Senior Researcher, General Genetics Corporation, Golden, CO, 80403.
1978 - 1981: Fish Pathologist, Wildlife Vaccines Inc., Wheat Ridge, CO, 80033.
1968 - 1971: Staff Sargent, United States Army.

PRIVATE CONSULTING:

July - August 1992: Special Consultant on Aquaculture, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD.
August 1985: Aquaculture Consultant, USAID, Quito, Ecuador.
November 1981 and 1983: International Center for Aquaculture, Auburn University, Auburn, AL.

CERTIFICATIONS:

1989 Fisheries Pathologist, Certificate # 54
1988 Fisheries Scientist, Certificate # 1910

PUBLICATIONS:

Taylor, P. W. (In press). Multiple Antimicrobial Resistence in a Chronic Bacteremia of Koi (*Cyprinus carpio koi*). North American Journal of Aquaculture.

Taylor, P. W. and J. R. Winton. (In press). Optimization of Nested Polymerase Chain Reaction Assays for Identification of *Aeromonas salmonicida, Yersinia ruckeri* and *Flavobacterium psychrophilum*. Journal of Aquatic Animal Health.

Taylor, P. W. and S. D. Roberts. 1999. Clove Oil: An Alternative Anaesthetic for Aquaculture. North American Journal of Aquaculture 61: 150 - 155.

Taylor, P. W., J. E. Crawford and E. B. Shotts. 1995. Comparison of Two Biochemical Test Systems with Conventional Methods for the Identification of Bacteria Pathogenic to Warmwater Fish. Journal of Aquatic Animal Health 7:312 - 317.

Jenkins, J. A. and **P. W. Taylor**. 1995. An Alternative Bacteriological Medium for the Isolation of *Aeromonas*. Journal of Wildlife Diseases 31:272 - 275.

Starliper, C. E., R. K. Cooper, E. B. Shotts and **P. W. Taylor**. 1993. Plasmid-Mediated Resistance of *Edwardsiella ictaluri*. Journal of Aquatic Animal Health 5: 1 - 8.

Cooper, R. K., C. E. Starliper, E. B. Shotts and **P. W. Taylor**. 1993. Comparison of Plasmids Isolated from Romet-30-Resistant *Edwardsiella ictaluri* and Tribrissen-Resistant *Escherichia coli*. Journal of Aquatic Animal Health 5: 9 - 15.

Taylor, P. W. 1992. Fish-eating Birds as Vectors for *Edwardsiella ictaluri* on Commercial Catfish Ponds in Mississippi. Journal of Aquatic Animal Health 4: 240 - 243.

Jack, S. A., **P. W. Taylor**, M. D. Crosby, J. Freund, J. R. MacMillan and R. M. Durborow. 1992. Summary of Bacterial Isolates from Farm-reared Channel Catfish (1979 - 1988). Journal of Veterinary Diagnostic Investigations 4: 193 - 195.

Taylor, P. W., and M. R. Johnson. 1991. Antibiotic Resistance in *Edwardsiella ictaluri*. American Fisheries Society, Fish Health Section, Newsletter Vol 19(2): 3 - 4.

Durborow, R. M., **P. W. Taylor**, M. D. Crosby and T. D. Santucci. 1991. Fish Mortality in the Mississippi Catfish Farming Industry in 1988: Causes and Treatments. Journal of Wildlife Diseases 27: 144 - 147.

Freund, J. D., R. M. Durborow, J. R. MacMillan, M. D. Crosby, T. L. Wellborn, **P. W. Taylor** and T. E. Schwedler. 1990. Utilizing Diagnostic Laboratory Records to Monitor the Occurrence of ESC in Channel Catfish in Mississippi. Journal of Aquatic Animal Health 2: 207 - 211.

Taylor, P. W. 1987. Antibiotic Resistance to Romet-30 in Bacterial Infections of Catfish. American Fisheries Society, Fish Health Section, Newsletter Vol 15(2): 4.

Taylor, P. W., W. A. Rogers and R. A. Dunham. 1884. Comparison of Hemoglobins and Hematocrits of Blue, Brown Bullhead, Channel, White, Crossbred and Hybrid Catfishes. Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agents 38: 374 - 382.

Taylor, P. W. 1978. *Macroderoides trilobatus* sp. n. (Digenea: Macroderoididae) from the Bowfin, *Amia calva*, and an Emendation of the Genus. Journal of Parasitology 64: 393 - 394.

Taylor, P. W. 1977. Isolation and Experimental Infection of Free-living Amebae in Freshwater Fishes. Journal of Parasitology 63: 232 - 237.

Taylor, P. W. 1979. Manual in Spanish "La Salud de los Peces", International Center for Aquaculture, Auburn, AL. 103pp.