Project 35055

Response to ISRP comments

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

Sponsor USFWS

I) ***It would help the project to place this work in the context of RPAs under the FCRPS BiOp; the presentation had these listed; they should be added to the proposal?***

The RPA's for this proposal are as listed in the budget section of the proposal, which are:

RPA 1: The Action Agencies, coordinating with NMFS and USFWS, shall develop 1- and 5-year plans to implement specific measures in hydro, habitat, hatcheries, harvest, research, monitoring, and evaluation needed to meet and evaluate the performance standards contained in this biological opinion.

Project relation: This is a USFWS proposed project which provides research, monitoring, and evaluation of hydro resources and habitat.

RPA 152: The Action Agencies shall coordinate their efforts and support offsite habitat enhancement measures undertaken by other Federal agencies, states, Tribes, and local governments by the following:

1. Supporting development of state or Tribal 303(d) lists and TMDLs by sharing water quality and biological monitoring information, project reports and data from existing programs, and subbasin or watershed assessment products.
2. Participating, as appropriate, in TMDL coordination or consultation meetings or work groups.
3. Using or building on existing data management structures, so all agencies will share water quality and habitat, data, databases, data management, and quality assurance.
4. Participating in the NWPPC's Provincial Review meetings and Subbasin Assessment and Planning efforts, including work groups.
5. Sharing technical expertise and training with Federal, state, Tribal, regional, and local entities (such as watershed councils or private landowners).
6. Leveraging funding resources through cooperative projects, agreements and policy development (e.g., cooperation on a whole-river temperature or water quality monitoring or modeling project).

Project relation: This is a Federal agency proposed project that will aid in water quality and biological monitoring information for subbasin and watershed assessments.

RPA 155: BPA, working with BOR, the Corps, EPA, USGS, shall develop a program to 1) identify mainstem habitat sampling reaches, survey conditions, describe cause-and-effect relationships, and identify research needs; 2) develop improvement plans for all mainstem reaches; and 3) initiate improvements in three mainstem reaches. Results shall be reported annually.

Project relation: Project will help to determine cause and effect relationships and identify research needs for improvement plans.

RPA 198: The Action Agencies, in coordination with NMFS, USFWS, and other Federal agencies, NWPPC, states, and Tribes, shell develop a common data management system for fish populations, water quality, and habitat data.

Project relation: This is a USFWS project that will provide data for management of water quality and habitat use and restoration.

II) ***What is the reason for sampling in 10 locations? How did you derive this number of sites?...How many samples will be taken from the ten sample sites, over which period of time?***

The selection of how many sites was simply geographical. As stated in the proposal, sites were selected below the inputs of named creeks in the system, below the facility, and two sites at the headwaters. I derived this number of sites first by assessing the time and energy it would take for me to collect, run tests, store, and analyze the samples. Then I looked at the watershed and assessed what would be reasonable under these constraints. I then decided that although it would be nice to take samples in more locations it was just not going to be logistically plausible. So I searched for a deciding factor, such as a smaller tributary, and found that if I used that criteria I would be within my constraints. Even then, I added two sights in the upper most reaches to provide a baseline of bacteria fauna in the water, then I added the one below the outflow of the facility because I had already collected some water samples there. Sampling is to occur as stated in the proposal, biweekly from April to October. At this point it is expected that one sample per site is adequate, the question being how much volume is necessary, and not how many. Although, as stated below, the project's budget should still cover an increase in sampling sites and number of samples. (Note: Read response V)

III) ***The proposal could be clearer on how presence of bacteria types will be linked to the level of ecosystem health.***

Bacterial assemblages are the result of the availability of a resource/nutrients, temperature, multiplication rates, and competition/predation of bacteria and aquatic organisms. Bacterial assemblages have been shown to have correlations of bacterial numbers versus the numbers of aquatic organisms or inputs. By observing these associations in action, an individual can watch patterns occurring that correlate with changes in a watershed. For example, Leff et al, 1998 and Lemke and Leff, 1999, suggested that there is a correlation between the numbers of *Burkholderia cepacia* and leaf matter. They found that *B. cepacia* was in high abundance on leaves. They also found that during the autumn, when leaf litter occurs most, water samples had an increase in *B. cepacia* numbers. This suggests that there is a direct correlation between leaf litter inputs and *B. cepacia* population numbers. From an ecosystem health perspective, relative to the type of ecosystem you are in, an increase in *B. cepacia* could indicate the amount of vegetation that exists in a watershed. Thus, you would expect to find high counts of *B. cepacia* in the autumn and low counts in the winter and intermediate counts during the spring and summer. On the contrary, if you found lower counts and no difference or slightly different amounts in population for seasonal adjustments, this would indicate a lack of vegetative cover. As seen in my proposal, it would be expected that we would find a lesser concentration of *B. cepacia* in the logging areas versus a higher concentration in the lower reaches of the watershed. These principles and theories apply to the other bacteria on the list. If you find more *Proteus vulgaris*, it would indicate more fecal material; more *Lampropedia spp*., more organically rich soils; more *Bacillus thuringiensis*, more insecticide use; etc. Thus, giving us a look at what is being used and inputs on the system.

Once the patterns are observed, these sequences of change can be determined, compared to environment and land use variables, and used to predict outcomes of bacterial populations based on changes or alterations to the watershed. By this manner we would be able to take the observation data, given each season and the environment from which they were collected, and connect the bacterial population fluctuations to given variables. Then we could form models that would allow us to predict the outcome in a given situation. This would allow us to see the sequences of events that must occur before a desired event can occur.

As a hypothetical example to increase salmon populations. First, we find that in a healthy watershed:

A) there is an increase of *Burkholderia cepacia* (indicator of allochthonous material) that drops off in the winter;

B) an increase of *Flavobacterium* spp.(associated with cold temperatures) in the fall that increases through the winter and slowly drops off until the fall;

C) an increase of *Xanthomonas* spp. (indicator of plants) in the spring which gradually drops through the year;

D) there are no indications that *Proteus vulgaris* (indicator of fecal material) exists except in the early fall;

E) there is a significant presence of *Pseudomonas aeruginosa* (indicator of organically rich soils) all year except in the winter when temperatures are below its range;

F) a spike of *Bacillus thuringiensis* (indicator of insects) in the late summer that bottoms out in late winter;

G) there is a greater presence of *Pseudomonas pickettii* (indicator of natural materials) in the summer and fall than there is in the winter and early spring.

H) *Pseudomonas putida* (indicator of mineral enrichments) is present all year, but usually is in limited numbers.

Upon taking a few water samples at periodic times through the year in a watershed of interest, we find that the watershed has a small presence of *B. cepacia* that barely shows an increase in the fall, no trace of *Xanthomonas* except in the spring and early summer when there is a minor presence, a strong population of *Pr. vulgaris* all year except winter, *Ps. putida* has very strong presence, *Ps. pickettii* is in small numbers, and *B. thuringiensis* has a limited population all year. After this discovery we make the assessment that there is limited biomass in the stream (specifically decaying organic matter), limited vegetation, too much fecal material (probable cause by humans), and mineral enriched soils.

Looking at the bacterial fauna and its cycles at a healthy system, we determine that the appropriate remedy is to: 1) increase the decaying or dead biomass in the autumn (increasing *Ps. pickettii*), creating food for invertebrate larvae and fish and slows the stream so that vegetation can more easily grow, 2) then in the late winter and spring, increase vegetation (which will increase are *Xanthomonas* levels) so that nutrients in the soil are created (increasing *Ps. aeruginosa*) by breaking down unusable ions and elements (decreasing *Ps. putida*) so that they are absorbed or altered into usable resources, while at the same time slowing runoff and increasing soil water retention; 3) this in turn converts the fecal material into usable forms (decreasing *Pr. vulgaris*) and promotes an increase in biomass (increasing *Ps. pickettii*); 4) lastly macroinvertebrate larvae feed on the allochthonous inputs (increasing *B. cepacia*) and plants (increasing the insect population and a presence of *B. thuringiensis*) and are eaten by our fish species of interest, which in turn return yearly to lay eggs and die in our streams to aid in an increase in biomass and decaying matter in continuing the cycle (increasing *Ps. pickettii*).

IV) ***Without a control, this work will be limited to establishing a description of the ecosystem conditions in association with certain groups of bacteria, but the study will not generate understanding of processes by which these bacteria/conditions associations work.***

This was understood when the proposal was submitted. It was not the intention of this proposal to find out the processes by which these bacteria/conditions associations work. Some of these processes have already been researched and continued research is exploring them in laboratory settings. The proposed project intends to look at the relationships of the bacterial fauna to other aquatic organisms, fish, and the environment. By observing these relationships, a correlation to the numbers of aquatic conditions and to bacterial numbers will greatly enhance our understanding of the intricacies of aquatic ecosystems.

V) ***We note that the investigator did not provide further evidence of progress on development of the statistical design; consequently, we again request a response describing a statistically sound study design.***

After discussing the project at great lengths with a statistician over project designs, a few main things came to issue. First, the ISRP should keep in mind that the project was submitted as an exploratory project. Hence the wording as "may aid," or " hypothetically possible," or "may make it possible." These words were explicitly placed throughout the proposal to emphasize that there are unknowns that need to be explored before statistically valid conclusions can be drawn. The crux of the project is having the funding made available so that the preliminary work can be accomplished. Preliminary meaning the planning and design phase.

Thus, as explained in the proposal, to begin sampling for monitoring purposes, I first must find out what organisms are present in the watershed by isolation on media. Then I can send pure cultures of bacteria from the creek to MicrobialID for identification. Once the organisms are identified, development of the primers that are necessary for monitoring to occur. After the primers have been developed, the primers can then be tested for levels of detection of bacterial DNA. Once this is accomplished, determination of the likelihood of detecting differences or effects of the sampling can measured. This information is necessary to determine the measurement of error, degree of confidence, effect size, and power needed to reject the null hypotheses. The hypotheses being:

1. H_o = There is no difference between land use and the indicator bacteria present.
   H_a = There is a difference between land use and the indicator bacteria present.
2. H_o = There is no difference between the environmental parameters and the indicator bacteria present.
   H_a = There is a difference between the environmental parameters and the indicator bacteria present.
3. H_o = The bacterial indicators's presence does not change seasonally.
   H_a = The bacterial indicators's presence changes seasonally.

The statistical measurements (listed in line above hypotheses) must be determined before any project design is valid. Noting the complexities of the project and in anticipation to changes in the sampling design, the budget submitted should still cover for a doubling of sample areas and a tripling of samples taken in those areas. Even if these alterations were to occur, sampling biweekly will still happen.

There were a couple of questions that were asked at the presentation that were not adequately answered due to the time constraints. Here's another attempt at answering one of thsse questions. Other questions are answered above.

VI*) Why use bacteria as indicators when there are already studies that show that the use of macroinvertebrates work well as indicators of water quality? Why search out even smaller organisms?*

The benefits of using bacteria as indicators of water quality and for watershed assessment surpass the previous abilities of current methods and are far less expensive than procedures that involve invertebrate counts, chemical tests, aerial land use photos and maps, and the use of amphibians and fish. These previous methods of measuring water quality and watershed health not only tend to be expensive, but also to be time consuming and only provide data for one segment or part of a watershed ecosystem.

Most often, these tests are used for indicating pollution points and occurances. Such water quality methods provide little, if any knowledge, or ability to assess the biological integrity of a watershed. Bacterial indicators would allow us to assess not only water quality issues and pollution points, but also allow a measurement of the biological integrity of a system. This is derived from the knowledge of the associations between bacteria and their ecosystems. Bacteria have direct and indirect associations with organic matter, organisms, and environmental factors such as temperature, salinity, pollution points, etc.

As an example, the lining of intestinal tracts of animals is covered with a fauna of bacteria. These bacteria act together to aid the breakdown of organic matter, exchange unusable ions and elements for useable nutrients that the intestine can absorb. While at the same time, the dynamics of their associations with each other maintain an equilibrium so as to keep the specie populations in check with each other, thereby the organism remains healthy. Perturbations and introductions to the system are usually kept under check or removed by these associations. In extreme situations, the system can become overwhelmed, in shock, or destroyed beyond the natural ability of the fauna to maintain homeostasis. At this point the system is sick, and unless steps are allowed to occur naturally or are taken to replace the fauna to its homeostatic functioning, the system does not perform as well as before and/or might not be able to perform its functions at all.

This is the same theory that applies to the watershed and its associations of bacteria with other aquatic life. The theory being that a watershed under natural conditions will maintain homeostasis at any given time if it is left to itself. Due to the presence of mankind utilizing natural resources and developing in areas that were once more natural, the question remains on whether such changes are altering the natural capabilities of ecosystems to where the bacterial fauna has changed to the point where steps that were once used to restore habitat to promote desired fish stocks, overestimate the ability of man to restore the balance of nature and may prove that such changes in habitat have removed associations that are necessary for selected stocks to exist. In other words, projects that are trying to improve fish habitat may well be unnecessary because of two reasons: 1) with the conditions the way they are, these fish stocks may not be possible to recover and other fisheries may be more adapted to the current conditions and may prove to be the most fit, or 2) in trying to restore the environment to past conditions we may be inhibiting nature from utilizing natural processes that restore the balance necessary for these stocks and species to exist. This includes the associations of all organisms in the watershed, and just because we alter the watershed to what we think it should look like, does not mean that the watershed will be as productive or be able to maintain past populations or species.

Therefore, using bacterial indicators will reduce the cost and time used for analyzing water quality and will provide insight and knowledge to the biological integrity of watersheds.

References

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., L. G. Leff, 1999, *Bacterial populations in an anthropogenically disturbed stream: Comparison of different seasons*, Microbial Ecology 38: 234-243