January 29, 2003

Hannibal Bolton
Division of Fish and Wildlife Management Assistance
U.S. Fish and Wildlife Service
4401 N. Fairfax Dr., #840
Arlington, VA 22203

Re: Wild Fish Habitat Initiative semiannual progress report

Dear Hannibal:

I'm pleased to submit the first semiannual report describing activities for the Wild Fish Habitat Initiative during July to December 2002. The purpose of the Initiative is to enhance the success of riparian projects conducted through the Partners for Fish and Wildlife Program. The Initiative involves two activities: conducting targeted research to assist Partners fish habitat restoration projects, and implementing a vigorous information-transfer program to provide technical results to those who plan and carry out Partners projects.

To date, our most visible product is the website developed for the information-transfer project (http://water.montana.edu/wildfish/). The site contains information on all aspects of the Initiative, including summaries of research projects, bibliographic information related to fish habitat restoration, and a case histories database. Although much information has yet to be obtained for the case histories database, I encourage you to take a look at the site. We hope this dynamic website and the other research projects associated with the Initiative will provide useful tools for Partners Program personnel.

If you have questions regarding this project, please contact me. If you would prefer to speak directly with Gretchen, she will be visiting Washington D.C. March 17-19, 2003 and will be available to meet with you. We look forward to the coming year and the opportunity to collect additional information.

Best regards,

Michelle D. White
Program Administrator

WILD FISH HABITAT INITIATIVE

SEMIANNUAL REPORT

July 1, 2002 – December 31, 2002

Submitted by:
Montana Water Center
Montana State University – Bozeman

Submitted to:
Division of Fish and Wildlife Management Assistance
U.S. Fish and Wildlife Service

Background

Habitat degradation is one of the principal reasons for the listing of wild fish as "threatened" or "endangered" under the Federal Endangered Species Act. Habitat degradation can exacerbate detrimental effects of fish predators, exotic competitors, and diseases such as whirling disease. In addition, land values are diminished by habitat degradation and the subsequent loss of wild fish populations. Private landowners forego economic opportunities when land uses are restricted and resources are directed toward fish restoration. In recent years, many techniques of fish habitat enhancement have been implemented, but their long-term efficacy is not well understood because little or no evaluation and monitoring have been conducted.

The Partners for Fish and Wildlife Program, administered by the U.S. Fish and Wildlife Service, is a critical national effort to restore important fish and wildlife habitat. This is a voluntary program that works with private landowners to restore habitat on their lands. ***The purpose of the Wild Fish Habitat Initiative is to enhance the success of riparian projects conducted through the Partners for Fish and Wildlife Program***. The Initiative involves two activities: conducting targeted research to assist Partners fish habitat restoration projects, and implementing a vigorous information-transfer program to provide technical results to those who plan and carry out Partners projects. Progress on each of the four projects is described below.

Evaluation of Entrainment Losses of Westslope Cutthroat Trout at Private Irrigation Diversions on Skalkaho Creek, Montana

Graduate Student: Steve Gale
Principal Investigators: Al Zale and Tom McMahon
Collaborator: Christopher G. Clancy
Montana Department of Fish, Wildlife and Parks

Background

Skalkaho Creek is a 40.4-km long tributary of the Bitterroot River located in southwest Montana. The Bitterroot flows 134 km through irrigated farm and ranch land to its confluence with the Clark Fork River near Missoula, Montana. Five major diversions and numerous smaller canals remove water from the river during irrigation season. Many tributaries of the Bitterroot River are also diverted for irrigation during the summer months and contribute little streamflow to the river during that time. Both the mainstem of the Bitterroot River and its tributaries are therefore chronically dewatered during the irrigation season.

Skalkaho Creek supports a healthy population of westslope cutthroat trout (*Oncorhynchus clarki lewisi*), along with brook trout, brown trout, bull trout, mountain whitefish, redside shiner, and slimy sculpin. This study will examine eight lowhead dams on lower Skalkaho Creek that are believed to divert downstream migrant westslope cutthroat trout into irrigation canals. Both post-spawn adults migrating back to the Bitterroot River and juveniles emigrating downstream from nursery reaches of Skalkaho Creek and its tributaries are likely entrained and become trapped and die in the irrigation

canal system, thereby resulting in a net loss to the population. Private landowners and irrigators in the drainage have expressed concern over this possible loss and will be installing fish screens at the diversions to preclude any such losses. The current magnitude and effects of entrainment by eight diversions on the westslope cutthroat trout population prior to the installation of fish screens, as well as the efficiency of the screens after installation, will be quantified. This study will provide beneficial information to project managers regarding the effectiveness of fish screens and the prevention of fish loss due to irrigation diversions.

Objectives:

1. Quantify downstream migrating juvenile and adult westslope cutthroat trout entrained at irrigation diversions on Skalkaho Creek, before and after installation of fish screens.
2. Evaluate passage efficiency of fish screen structures at irrigation diversions in Western Montana.

Progress to Date

Work on the irrigation diversion project began in September 2002 with the beginning of the graduate student project associated with it. An extensive literature review of the life history and seasonal movements of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) was conducted. Field sampling protocols were researched and equipment was purchased.

Two trips to Skalkaho Creek were made in October 2002 to meet with biologists from Montana Fish Wildlife and Parks and the University of Montana. During these site visits, six irrigation diversion dams along Skalkaho Creek were investigated to learn more about fish entrainment problems and the proposed plans to install fish screens. One screen will be installed during fall 2003 by Montana Fish Wildlife and Parks; additional screens will be installed in subsequent years. Work on this project will focus on the collection of data before fish screen installment and the evaluation of westslope cutthroat populations after installment.

Future Activities

Equipment will field tested this spring. During spring and fall 2003, 30 adult and 50 juvenile westslope cutthroat trout will be fitted with radio-telemetry tags in spawning and nursery areas of upper Skalkaho Creek prior to their respective emigration periods. Their location and movements will be monitored several times per week to determine their success in bypassing irrigation diversions during their downstream migration to the Bitterroot River. The final fate of each individual will be determined (i.e., in-river mortality, canal mortality, in-river residence, or successful emigration to the Bitterroot). The process will be repeated during the second year of the study, after the installation of fish screens.

Tagged westslope cutthroat trout that successfully migrate to the Bitterroot River will be tracked to locate their winter habitat. Trapping will be used to estimate abundances for entrained and down-migrating juvenile westslope cutthroat trout that are too small to be

tagged. All screened and non-screened diversions will be electrofished to corroborate and supplement findings of trapping and telemetry experiments. In addition, temperature will be recorded at stations along Skalkaho Creek using HOBO temperature recorders set by the Montana Fish Wildlife and Parks. Flow for Skalkaho will be recorded at USGS gauging station #12346500. These data will be used to determine out-migration cues for westslope cutthroat trout.

Thermal Requirements of Westslope Cutthroat Trout

Graduate Student: Beth Bear
Principal Investigators: Tom McMahon and Al Zale
Collaborator: Bill Krise, Bozeman Fish Technology Center, USFWS

Background

Historically, westslope cutthroat trout (*Oncorhynchus clarki lewisi*) ranged widely over western Montana, Idaho, and portions of eastern Washington and Oregon. Like many other cutthroat and other native trout, westslope cutthroat trout now persist in only a small portion of their native range, and are listed as a "species of special concern" in Montana.

Leading causes for their decline are habitat degradation and displacement by non-native rainbow, brook, and brown trout. Water temperature is considered a key element in the abundance and distribution of cold water species like trout, yet the thermal requirements of westslope cutthroat trout, like many other native fishes, are largely unknown. In addition, increased water temperature is thought to favor non-natives in many cases, yet the effect of temperature on competition between westslope cutthroat and non-natives is unknown. Furthermore, hybridization between westslope cutthroat trout and non-native rainbow trout has resulted in a decline in populations of genetically pure westslopes. It is unclear what the thermal requirements of these hybrids are, as well as how the competitive interaction between hybrids, genetically pure westslope cutthroat trout, and non-natives is influenced by water temperature.

The goal of this laboratory study is to characterize the thermal biology of westslope cutthroat trout, specifically with respect to the lethal and optimal temperatures for this subspecies, and to compare its performance against a non-native competitor in sympatry and allopatry. We will use a laboratory design that we developed for thermal testing with bull trout (Selong et al. 2001). This design allows simultaneous assessment of fish growth and survival under many different thermal regimes over long time periods.

Objectives:

1. To define the upper lethal and optimal temperature ranges of westslope cutthroat trout.
2. To determine how temperature influences the competitive interactions with non-native species such as rainbow trout (*Oncorhynchus mykiss*).

3. To contrast how thermal requirements of westslope x rainbow trout hybrids compare with pure westslope cutthroat trout and pure rainbow trout as a means of assessing why hybrids between these two species have been so successful.

Progress to Date

Work on the westslope project began October 15th with the beginning of the graduate project associated with it. In the early stages most time was devoted to getting a new graduate student on board, as well as assisting with a similar experiment at the thermal testing facility using bull trout. This introduced the student to the state-of-the-art facility and protocols which will be used during the westslope cutthroat project.

In late October, 36 Onset Optic Stow-Away Temperature Loggers were purchased for use in monitoring test temperatures during the westslope thermal trials. These temperature loggers were calibrated using a known protocol, and will record temperatures in each tank at an interval of 15 minutes, with a +- 0.3° C degree of reliability.

Westslope cutthroat trout eggs were obtained last spring, and fry are being held at the Bozeman Fish Technology Center where our thermal testing facility is located. These fry are being reared in 4 separate tanks with a continuous water flow from a cold spring, keeping the water temperature around 8°C. Several other tanks in this building also use water directly from this cold spring, and water supply is often limited. In early December, cold water supply to other tanks in the building was accidentally increased for too long a duration, thus diverting water flow to the tanks containing the westslope fry. Without constant water flow, the fry had insufficient oxygen to stay healthy and alive, and mass mortalities resulted. To conduct a temperature trial, 1800 fish are required and there are currently only 1500 fish available. Therefore, it has become necessary to obtain additional westslope cutthroat young of the year (YOY) for use in this first temperature trial. It has been arranged to acquire 2000, 2.5 – 3 inch Roger's Lake YOY (the same stock as those being reared at the tech center) from Murray Springs Hatchery in Eureka, MT. These will be acquired in late January and acclimated to the tech center for 2 weeks, before use in the first temperature trial.

As a solution to the problem of diverted water flow, we have decided on purchasing low flow warning systems, which alert personnel if water levels drop below an acceptable point. The systems are estimated at $500 each with two needed (one on the thermal tanks and one on the rearing tanks). In the interim, the water flow to the rearing tanks has been changed to a mix of cold and warm water to ensure that in the event that one of these sources is diverted, there will still be sufficient water flow into the tanks; this action has raised the temperature of the water to 13°C which is acceptable rearing temperature for these fish.

Future Activities

A tentative schedule has been prepared outlining the upcoming 20 months with five, 90 day trials being performed within this time frame. Thermal tests will begin in February 2003 with the optimum growth temperature and ultimate incipient lethal temperature for westslope cutthroat trout to be determined using the acclimated chronic exposure method

(Selong et al. 2001). This study will also include four other thermal trials, to be outlined further in the research proposal which will be completed by May 2003. Results of the study will help guide protection and restoration efforts for this unique cutthroat trout.

Bacterial Coldwater Disease in Westslope Cutthroat Trout: Hatchery Epidemiology and Control

Principle Investigators: Eileen K. N. Ryce and Al Zale

Background

Bacterial coldwater disease, caused by the gram-negative bacterium *Flavobacterium psychrophilum*, is a septicemic infection that has caused significant losses of hatchery-reared salmonids worldwide. Currently, bacterial coldwater disease is the only disease found in State fish hatcheries in Montana. Increasingly, these hatcheries are being asked to help restoration programs for rare and sensitive species. The Washoe Park State Fish Hatchery in Anaconda, MT, is currently the only facility in the state producing westslope cutthroat trout suitable for restoration programs. However, the severity of bacterial coldwater disease at this hatchery inhibits its use for westslope cutthroat trout restoration projects. Successful control of bacterial coldwater disease in the hatchery would help facilitate restoration of native westslope cutthroat trout in Montana. The source of bacterial coldwater disease at Washoe Park is unknown, as is the epidemiology of the bacterium. New control measures must be developed and tested expeditiously to ensure the success of fish restoration projects.

Many facets of the disease epidemiology make it difficult to manage and control. The disease affects all sizes of salmonids; however, juveniles are most susceptible to the disease (Branson 1995). The coldwater disease bacterium can be transmitted both horizontally (from fish to fish) and vertically (from adult to egg) (Brown et al. 1997) and has been observed in the ovarian fluid and on the surface of fish eggs even after disinfection (Rangdale et al. 1996; Brown et al. 1997). Transmission within and on the eggs is therefore a major concern in the control of this pathogen. In the absence of a clinical disease outbreak the bacterium is commonly isolated from the brain of fish. This reservoir of bacteria can also act as a potential source of a disease outbreak if triggered by a stress condition (Dalsgaard and Madsen 2000). The disease can be precipitated by stress caused by general hatchery procedures including grading, handling, movement, change of feeding regimes and also by poor water quality and the presence of other diseases (Branson 1995). The bacterium is also able to survive outside the host making water a possible source of infection (Vatsos et al. 2001).

Typical clinical signs of bacterial coldwater disease include lethargy, dorsal skin erosion, ascites (accumulation of fluid in the peritoneal cavity), bilateral exopthalmia, very pale gills, and hemorrhagic vent with trailing mucoid casts. Concurrent problems, such as infestation with parasites, are common (Branson 1998). Internally, the most obvious finding in fish with this condition is splenomegaly, where the spleen can be 2 to 5 times its normal size and is usually friable in nature. The surrounding peritoneum and fat are usually red in color and kidneys may be pale and slightly swollen. Intestines often

contain yellow or white mucoid discharge and the terminal gut may be congested. Damage to the spleen can be seen histologically and the bacteria can be seen within the damaged tissue (Branson 1998). Signs associated with neurological disruptions can also occur including whirling behavior around the longitudinal axis, post cephalic protrusion of the cranium, spinal deformities, and loss of melanocyte control in the posterior body (also known as black-tail) (Kent et al. 1989; Meyers 1989). Microscopic features of the neurologic lesions include fibrous inflammation of the vertebral canal at the junction of the spinal cord and the medulla oblongata. The inflammation causes an upward compression of the anterior position of the spinal cord. Erosion and necrosis of vertebral bone and cartilage is also apparent (Meyers 1989). Many of the clinical signs associated with bacterial coldwater disease are also clinical signs of other fish diseases; therefore, a positive identification of the bacterium is necessary for confirmation of the disease.

Traditionally, oxytetracycline incorporated into fish food was the most common form of treatment for bacterial coldwater disease. The drug is usually effective in hatcheries experiencing the condition for the first time, but resistance towards this drug is developing (Branson 1995). The drug amoxycillin is now commonly used in Europe to control the disease; however, resistance to this drug is also increasing (Branson 1998). Although resistant bacteria are developing, oxytetracycline remains the treatment of choice in North America. Increasing incidence of the resistant bacteria and recurrent outbreaks of disease shortly after a treatment are continual problems, and no alternative treatments are currently available (Dalsgaard and Madsen 2000). A *F. psychrophilum* vaccine has been developed using the immunogenic outer membrane fraction of the bacteria; however, this vaccine is not yet commercially available and needs to be further investigated for viability and economical production on a large scale (Rahman et al. 2002). At present, the most effective form of disease control is to prevent outbreaks from occurring by reducing stress on the fish. The goal of this research is to better understand the ecology of *F. psychrophilum* in hatcheries so that control measures can be developed and tested.

Objectives:

1. To identify the source of *F. psychrophilum* at Washoe Park State Fish Hatchery;
2. To determine where in the hatchery production process *F. psychrophilum* is most prevalent and at what life stages westslope cutthroat trout are susceptible; and
3. To identify and evaluate measures at Washoe Park to eradicate or control the bacterium such that production, and consequently native species restoration efforts, are no longer hindered by the disease.

Progress to date

An in-depth literature review on the bacterium and the disease was completed. We determined the best methods to measure disease severity in test fish are with the use of clinical signs and microscopic pathology. Positive identification of the pathogen is also required. At present standard bacteriological isolation techniques are the only means of positively identifying the pathogen. These processes are time consuming but are the only option available at present to identify *F. psychrophilum*. A histology and bacteriology laboratory has been set up within the Montana Cooperative Fisheries Research Unit

primarily for use on this project. The laboratory is equipped with a tissue processor, an embedding station, a microtome, incubator and autoclave. Prior to acquiring this laboratory and the equipment, all histology and bacteriology work for the Unit had to be carried out at other institutions. The ability to conduct histology and bacteriology in the Unit laboratory allows for greater efficiency and flexibility within our fish health research program. In addition to acquiring the laboratory and the equipment, a part-time technician has been hired to aid in the operation of the laboratory.

Several visits have been made to Washoe Park to identify areas within the hatchery that may be potential sources or sinks of the pathogen. Observations on hatchery practices have been conducted to determine where within the production process changes could be made to reduce stress on the fish and subsequent outbreaks of the disease.

Future Activities
Samples are being collected from the hatchery to determine where the pathogen is most prolific and within what life stage of the fish the pathogen is present. Trials are soon to be set up to identify under what conditions the disease becomes most prolific. Stocking densities, water temperature and feeding regimes will be varied under controlled conditions to determine what conditions are most favorable for the survival of the fish in the presence of the pathogen.

Technology Transfer
Contributors: Michelle White, Cal Fraser, and Molly Boucher

Background
In recent years, many techniques regarding fish habitat enhancement and restoration have been implemented but project results generally have not been shared or exist only in "gray literature" where they are difficult to access. To address this problem, we are collating information on methods and results of various fish habitat restoration projects completed within the intermountain west (Idaho, Montana, Nevada, Wyoming, Utah, Colorado, eastern Washington, eastern Oregon, and eastern California). The information we are collecting includes narrative descriptions, project goals, restoration methods, project costs, landowner contributions, and monitoring data. We hope to augment the success of the Partners Program and other habitat restoration programs by providing useful bibliographic and case history information to land owners and project managers through a web-accessible database. By providing easily accessible information on effective fish habitat restoration and monitoring techniques, the overall project goal is to increase long-term effectiveness of such projects.

Objectives
1. Alert Partners biologists to the project and solicit their information needs.
2. Provide a web-accessible resource on fish habitat restoration, including bibliographic information of pertinent literature.
3. Provide a web-accessible case history database of fish habitat projects pertinent to Partners activities in the northwestern United States.

Progress to date

Partners Program personnel within the state of Montana were contacted in July 2002 and notified of the project. Initial meetings were conducted in October 2002 to discuss information needs of the biologists and to coordinate information exchange. The Partners Program has an extensive website with useful information; we are striving to augment the Partners site and produce a tool that does not duplicate previous efforts.

A template was designed for the website from July to October 2002; website design was completed in November after internal review (http://water.montana.edu/wildfish/). It provides background information on the Wild Fish Habitat Initiative and incorporates its three major components: current research projects, resource information, and a case histories database. Web pages for the research projects and resource information are complete; research projects outlined above are summarized on the website and will be updated as necessary.

Resource information on the website was developed during July through October 2002. Information on the site includes a searchable bibliography, a list of habitat restoration manuals, and links to pertinent on-line habitat restoration information. The on-line bibliography is a collation of information on various fish habitat restoration techniques and will facilitate information exchange among fisheries biologists and project managers. The list of habitat restoration manuals includes information relevant to the northwestern United States; each manual is available on the website in PDF format. Resource information will continue to be updated as new information becomes available.

During October and November 2002 the case histories database was developed. The searchable database will showcase fish habitat restoration projects completed within the northwestern United States and will include the following information: narrative descriptions, project goals, restoration methods, project costs, landowner contributions, photographs, and monitoring data. The intent of the database is to share information and learn from examples of previous restoration work. Currently there are three projects in the database that are accessible from the website. We will continue to gather information on additional projects utilizing a variety of restoration techniques.

Future Activities

Our efforts now are focusing on the collection of information on fish habitat restoration projects for the case histories database. Initially we will focus on projects within the state of Montana. After a variety of projects from Montana have been obtained and entered into the database, we will begin contacting Partners Program personnel from other states within the northwestern United States. As we begin collecting project information, we will make on-site visits, as necessary, to collect information and photographs and minimize the work required by project managers to supply us with information. We will continue to update the bibliographic information, and update and maintain the website as needed. By fall 2003 we intend to have case histories projects representing each state in the northwestern United States, at which time we will coordinate a formal review of the website by fisheries biologists and Partners Program personnel.

Project Personnel

Dr. Alexander Zale is the Principal Investigator. Dr. Zale is the Cooperative Fishery Research Unit Leader for Montana and an Affiliate Associate Professor in the Department of Ecology at Montana State University. Besides exercising overall leadership, he is the co-leader for the Bacterial Coldwater Disease project. Dr. Zale's research interests center on applied aquatic ecology and fisheries management.

Dr. Thomas McMahon is the Project Biologist. Dr. McMahon is an Associate Professor in the Ecology Department at Montana State University whose principal research interests are wild trout management, fish-habitat relationships, winter ecology, and conservation biology of salmonids. He is the leader of the Westslope Cutthroat Thermal Testing project and the Irrigation Diversions project.

Eileen Ryce, Post-Doctoral Associate in the Ecology Department at Montana State University, is co-leader for the Bacterial Coldwater Disease project. Dr. Ryce specializes in fish health issues.

William C. Fraser is directing the Technology Transfer project. Mr. Fraser is a fishery biologist who serves as Manager of the Wild Trout Research Laboratory at the Montana Water Center. His chief professional interests are salmonid ecology and culture.

Michelle D. White is a Water Quality Specialist with the Montana Water Center. She has degrees in general biology and marine science, and manages water quality projects for the Center. She is working on the Technology Transfer project and serving as project administrator for the Wild Fish Habitat Initiative.

Molly Boucher is a Program Specialist with the Montana Water Center. She has a degree in environmental studies and develops websites and databases for the Center. She is the website developer for the Wild Fish Habitat Initiative and works with William Fraser and Michelle White on the Technology Transfer project.

Beth Bear is a Graduate Research Assistant with the Montana Cooperative Fishery Research Unit at Montana State University. She is working with Dr. Thomas McMahon on the Westslope Cutthroat Thermal Testing project.

Steve Gale is a Graduate Research Assistant with the Montana Cooperative Fishery Research Unit at Montana State University. He is working with Dr. Alexander Zale and Dr. Thomas McMahon on the Irrigation Diversions project.

References

Branson, E. 1995. Rainbow trout fry syndrome. Fish Veterinary Journal 1:1-7.

Branson, E. 1998. Rainbow trout fry syndrome: an update. Fish Veterinary Journal 2:63-66.

Brown, L. L., W. T. Cox, and R. P. Levine. 1997. Evidence that the causal agent of bacterial coldwater disease *Flavobacterium psychrophilum* is transmitted within salmonid eggs. Diseases of Aquatic Organisms 29:213-218.

Dalsgaard, I., and L. Madsen. 2000. Bacterial pathogens in rainbow trout, *Oncorhynchus mykiss* (Walbaum), reared at Danish freshwater farms. Journal of Fish Diseases 23:199-209.

Kent, M. L., J. M. Groff, J. K. Morrison, W. T. Yasutake, and R. A. Holt. 1989. Spiral swimming behavior due to cranial and vertebral lesions associated with *Cytophaga psychrophila* infections in salmonid fishes. Diseases of Aquatic Organisms 6:11-16.

Meyers, T. E. 1989. Apparent chronic bacterial myeloencephalitis in hatchery-reared juvenile Coho salmon *Oncorhynchus kisutch* in Alaska. Diseases of Aquatic Organisms 6:217-219.

Rahman, M. H., A. Kuroda, J. M. Dijkstra, I. Kiryu, T. Nakanishi, and M. Ototake. 2002. The outer membrane fraction of *Flavobacterium psychrophilum* induces protective immunity in rainbow trout and ayu. Fish and Shellfish Immunology 12:169-179.

Rangdale, R. E., R. E. Richards, and D. J. Alderman. 1996. Isolation of *Cytophaga psychrophila*, causal agent of rainbow trout fry syndrome (RTFS) from reproductive fluids and egg surfaces of rainbow trout (*Oncorhynchus mykiss*). Bulletin of the European Association of Fish Pathologists 16:63-67.

Selong, J.H., T.E. McMahon, A.V.Zale, and F.T. Barrows. 2001. Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. Transactions of the American Fisheries Society 130:1026-1037.

Vatsos, I. N., Thompson, K. D., and A. Adams. 2001. Adhesion of the fish pathogen *Flavobacterium psychrophilum* to unfertilized eggs of rainbow trout (*Oncorhynchus mykiss*) and n-hexadecane. Letters in Applied Microbiology 33:178-182.