

**Arctic Char:
Development of Production Technologies
Suited to Water Resources in Appalachia**



**Final Project Report for
USDA/ARS Grant No. 59-1930-038**

**PREPARED BY:
THE CONSERVATION FUND**

FRESHWATER INSTITUTE

TABLE OF CONTENTS

I. EXECUTIVE SUMMARY	2
II. ACKNOWLEDGEMENTS	3
III. INTRODUCTION	4
A. NEED FOR RESEARCH	4
B. GOALS AND OBJECTIVES	4
IV. EARLY REARING OF ARCTIC CHAR	4
A. HATCHING SYSTEM	4
B. CULTURE OF CHAR EGGS AND FRY	6
C. GROWTH COMPARISON OF SMALL, MEDIUM AND LARGE CHAR	7
D. GROWTH IN RECIRCULATING SYSTEM	9
V. FISH HEALTH	10
A. STUDIES OF VIRAL PATHOGENS	10
1. <i>Susceptibility</i>	10
2. <i>Epidemiology</i>	11
B. STUDIES OF BACTERIAL PATHOGENS	13
1. <i>Susceptibility</i>	13
2. <i>Methods Development</i>	15
3. <i>Vaccination</i>	16
4. <i>Identification of a Rickettsia-like organism (RLO)</i>	16
5. <i>Epidemiology</i>	17
6. <i>Treatment</i>	18
VI. OUTCOMES	19
A. PEER-REVIEWED PUBLICATIONS	19
B. PRESENTATIONS WITH PUBLISHED PROCEEDINGS	22
C. PRESENTATIONS WITHOUT PROCEEDINGS	23
D. TECHNOLOGY TRANSFER	41
1. <i>Industry workshops and meetings</i>	41
2. <i>West Virginia Aqua</i>	42
3. <i>Quarterly reports</i>	42
4. <i>Telephone</i>	42
E. THESES AND DISSERTATIONS RESULTING FROM THIS PROJECT	42
F. COLLABORATORS	43

I. Executive Summary

In 1996, The Conservation Fund's Freshwater Institute began a five-year project, funded by the U.S. Department of Agriculture, to investigate the potential for the culture of Arctic char (*Salvelinus alpinus*) in the Appalachian region of the United States. The project, "Arctic char: Development of production technologies suited to water resources in Appalachia", began in September 1996, and was completed in September 2001.

The goal of this project was to promote the expansion and diversification of the United States cold-water aquaculture industry through development of Arctic char, a species suited to high volume ground-water resources common to Appalachia. To reach this goal, the Freshwater Institute identified and solved many husbandry constraints to Arctic char production within the water resources of Appalachia.

This project funded at least 15 manuscripts in peer-reviewed journals (including papers currently in press and in review). At least seven presentations with published proceedings and 49 presentations (including industry workshops and state aquaculture meetings) without published proceedings resulted from this work. Areas of work included:

- Culture of Arctic char eggs, fry and fingerlings
- Development of a system for early rearing of Arctic char
- Growth of char in flow-through and recirculating systems
- Studies of viral pathogens
- Studies of bacterial pathogens, including a "new" rickettsial-like-organism
- Vaccination of Arctic char against furunculosis
- Oxytetracycline residues in recirculating systems
- The effect of density and pathogen concentration on disease dynamics

This project created the opportunity for a number of collaborative research projects and relationships with industry that have resulted in significant advancement of char culture in the water supplies characteristic of the Appalachian region. During this project, the Freshwater Institute designed and tested a hatching system for early rearing of Arctic char. This system design is not only in use at the Freshwater Institute. It has been transferred to, and is in use at, commercial and government char and rainbow trout hatcheries in the United States. Early rearing of Arctic char in the warmer water conditions typical of Appalachian groundwater (12°C to 15°C as compared to 6°C to 8°C in Canada) was also studied and resulted in recommendations that were published and were passed directly on to U.S. char producers. Other study areas that have had direct impact on the aquaculture industry include the use of vaccination to protect char against furunculosis, results from epidemiology studies that were integrated with presentations on biosecurity given at industry meetings and work on susceptibility of char to pathogens that has helped producers anticipate, and plan for, potential fish health problems.

II. Acknowledgements

This report was prepared by The Conservation Fund's Freshwater Institute in December 2001, and was submitted to Dr. William Hershberger, ADODR, USDA-ARS National Center for Cool- and Cold-water Aquaculture as a final summary report, as required under 7CFR 3015.

The material is based upon work supported by the U.S. Department of Agriculture, Agricultural Research Service, under Agreement No. 59-1930-6-038. Any opinions, results, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

This report was developed in response to a request from the U.S. Department of Agriculture, Agricultural Research Service. Unless the material is from a peer-reviewed journal, or permission for use of non-peer-reviewed material is received from the Freshwater Institute, information contained herein is not intended to be quoted or cited in scientific literature. Inclusion of original data or information in this report is not intended to preclude its copyrighted publication in peer-reviewed scientific journals. Any experimental protocols or methods described herein involving live animals were in compliance with Animal Welfare Act (9CFR) requirements and have been approved by the Freshwater Institute Animal Care and Use Committee.

We would like to acknowledge all of the contributions made by current and past employees of the Freshwater Institute and affiliated contractors.

The citation for this report is:

Bebak-Williams, J. 2001. Final Project Report for USDA/ARS Grant No. 59-1930-6-038, *Arctic Char: Development of Production Technologies Suited to Water Resources in Appalachia*. The Conservation Fund's Freshwater Institute, Shepherdstown, WV, 43 pages.

III. Introduction

A. Need for research

Arctic char production is a new and rapidly expanding segment of the world aquaculture industry. Production of Arctic char has great economic potential within the water resources of the Appalachian region. Researchers in Canada, Iceland and Norway are investigating Arctic char production, but under significantly different culture environments than are common in Appalachia. No current research base exists in the United States for Arctic char production. This project focused on primary technological and biological constraints to Arctic char culture outside the natural range of this species.

B. Goals and objectives

The goal of this project was to promote the expansion and diversification of the United States cold-water aquaculture industry through the sustainable development of high volume, under-utilized water resources common to Appalachia. The objectives were:

- 1) Develop and evaluate optimal production technologies and engineered systems that will be required for producing Arctic char seed stock in Appalachian water resources.
- 2) Identify and solve husbandry constraints to Arctic char production within the water resources of Appalachia.

IV. Early rearing of Arctic char

A. Hatching system

- A recirculating system was designed and installed to incubate a maximum of 160,000-eyed eggs per stocking (Fig. 1). The incubation system contains two Heath-Tecna 8-stack incubators (Marisource, Tacoma, WA). Each 8-stack incubator is capable of incubating about 80,000 eggs on a flow of about 5 gal/min. An insulated cabinet encloses each 8-stack incubator in order to minimize temperature loss and any odors generated during formalin treatments, which are not typically required with eyed eggs (Fig. 2). This recirculating system contains two 1-hp pumps, which are plumbed in parallel. One pump is used to recirculate water at a flow rate of about 50 GPM while the other pump is held as a back up. The two pumps are hooked up to an automatic pump control unit to (1) allow for automatic transfer in case of failure of the lead pump and (2) connect to a float switch in the pump sump to alarm and turn ‘off’ the pumps in the event of low water levels. Make-up water addition to the pump sump is set manually with a dedicated valve at about 1 gal/min. However, a float valve is also installed in the pump sump to allow for automatic make-up water addition, as required. A

recirculating flow of about 200 L/min (50 gpm) is pumped through two air-cooled chillers (plumbed in series). Each chiller uses a titanium heat exchanger and a digital thermostat to maintain the hatching system at 6-12°C when a 1 gpm make-up water flow (at 12°C) is added to the system. This entire flow is pumped through a pleated cartridge filter with a 100µm cartridge. The flow is then split and approximately 40 L/min (10 gal/min) of the recirculating flow (5 gpm per 8-stack) is passed through a UV filter to disinfect the flow before it enters the two 8-stack incubators – the amount of flow depends on how many incubators are in use. The remaining ~ 160 L/min (40 gal/min) is by-passed around the incubators and can be directed either to an 8” diameter aeration column or it can be by-passed directly to the pump sump. Although aeration is necessary, in practice the amount of water flowing through the aeration column should be minimized to reduce over-stripping dissolved carbon dioxide from the water, which increases the precipitation of limestone (calcium carbonate) scale. For this reason, in future installation we recommend that a device that creates an electro-magnetic field that polarizes the particles in water should be installed to treat the entire 50 gal/min of recirculating flow. The pump pressure is monitored by a pressure switch, which signals an alarm if recirculating water pressure drops below about 15 psi. The water temperature is also monitored and an alarm is signaled if the water temperature exceeds a preset limit. An alarm is also signaled if either of the chillers shut down due to low coolant levels.

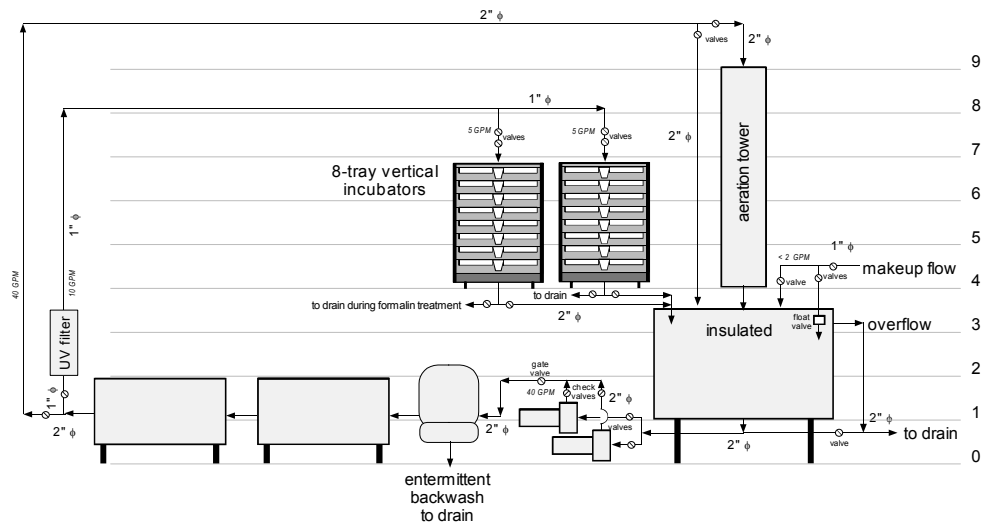


Figure 1. A process flow diagram of the recirculating ‘Hatching System’ developed at the Freshwater Institute. Note that the illustration is not to scale, component placements are not exact, and elevations are only approximate.

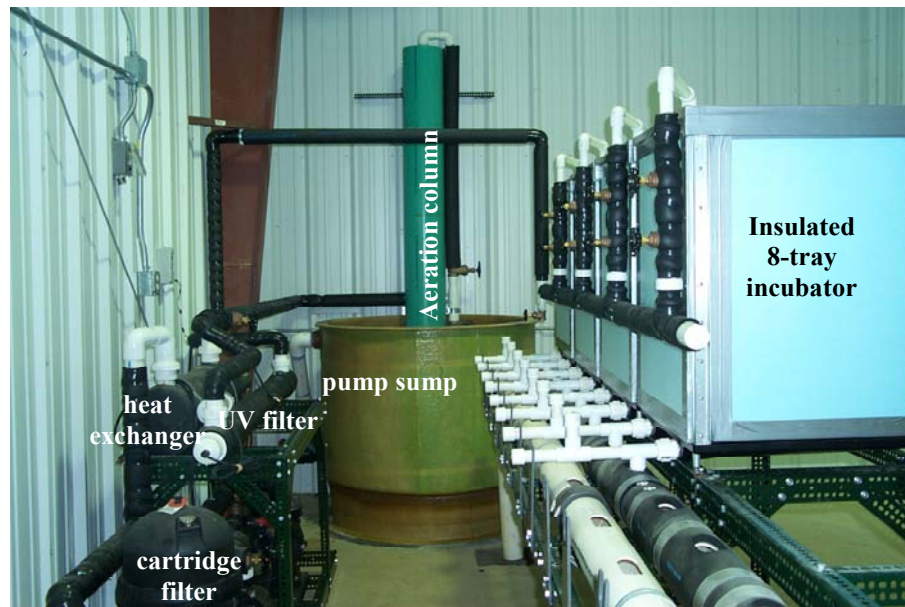


Figure 2. A similar recirculating hatching system (except containing two more 8-tray incubators) is in operation at the Mingo County Redevelopment Authority Hatchery in Delbarton, WV.

B. Culture of char eggs and fry

- **Bebak, J., J.A. Hankins and S.T. Summerfelt. 2000. Effect of water temperature on survival of eyed eggs and alevins of Arctic char. *North American Journal of Aquaculture* 62:139-143.** We determined hatching success and post hatch survival of Labrador (Fraser river, Canada) and Nauyuk (Yukon, Canada) strains of Arctic char *Salvelinus alpinus*, when incubated from the eyed egg stage to 90 d post hatch at temperatures ranging from 6.0°C to 12.0°C. Hatching success was 90% or more at all temperature regimes tested. For groups incubated, hatched, and reared at temperatures below 12°C, the probability of post hatch survival was 0.78-0.97. At a constant temperature of 12°C, the probability of post hatch survival was 0.65-0.73.
- In November/December of each year of this project, specific-pathogen-free eyed Arctic char eggs were imported from suppliers in Canada. Each year, eggs were incubated and hatched in Heath incubator trays in a chilled, recirculating hatching system designed and built by the Freshwater Institute. After eggs hatched, and fry were ready to start feeding, alevins (non-feeding fry) were transferred to 4' (1.2 m) diameter round tanks (flow through conditions) and cultured at a water depth of about 6" (15 cm). Degree of swimming activity, size of yolk sac and degree of lateral skin pigmentation were used to determine the readiness of the alevin to be

transferred. For each cohort, date of 50% hatch, date moved from hatching trays to fry tanks, percent survival to hatch and percent survival to 1000 ATU (ATU = Σ [culture temperature ($^{\circ}$ C) x days at culture temperature]) were recorded (Table 1). Time at 50% hatch was usually at 450 to 500 ATUs. Fry were moved from the incubator to fry tanks at about 150 ATU post-hatch. Hatching success from year to year averaged above 95%. Percent survival to 1000 ATU post hatch ranged from 74% to 97%, with a non-weighted average of 84%.

C. Growth comparison of small, medium and large char

- Char can be successfully incubated and hatched at higher temperatures than they experience naturally. It is unclear whether hatching and growth at these higher temperatures result in any long-term negative effects on growth rate. From July, 1997, to December, 1997, Freshwater Institute staff compared the growth of Labrador strain char (hatched in December, 1996) that had been incubated and hatched at three different temperature regimes and then divided into three size classes at the fingerling stage. Temperature treatments were 6.5-6.5-11.5, 8.5-8.5-11.5, and 9.5-9.5-11.5 (see Table 1 for explanation of temperature treatments). D.O. was maintained at 7.6 mg/L to 11.7 mg/L. Approximately 200 days post-hatch, each temperature group was size sorted with bar graders into three size classes qualitatively representing the upper third (LARGE), the middle third (MIDDLE) and the bottom third (SMALL) of each temperature treatment creating a 3x3 design randomly assigned across nine rearing tanks. For each size class, the coefficient of variation was between 25% and 35%. Fish loadings (weight per unit volume of flow) were maintained above 30 kg/m³ and below 100 kg/m³ by manipulating numbers of fish in each tank through random cropping of each tank after size and weight data collection. Five monthly samples provided fork lengths to the nearest mm for each rearing tank. Survival was excellent, well over 90% from hatch for all treatments. Water quality was near optimum and constant for all tanks through a shared manifold supply. Feed was supplied by pendulum demand feeder supplemented by hand feeding to satiation and was not limiting. Growth analysis showed that even though each size class was starting out at different sizes, the slopes of the lines were almost identical. Therefore, the early temperatures and early size sorting had no measurable effect on growth rates. The practical value of this information is recognition that the largest fish within a cohort may not have any genetic or other advantage over the rest of the population other than a brief and early step-in-size advantage that provided a head start. Over a period of five months, there was no sustained advantage demonstrated and perhaps more importantly, no apparent harm. The 60-90 day size stage advantage from the warmest fish to the coldest fish creates a real market spread and a management challenge. It may be that the common practices of selecting broodstock from only the largest fish in the cohort or conversely, culling the bottom third of the population out as runts is not borne out by the growth rate data. This may suggest that we are really unintentionally selecting for some characteristic of early hatching or some very brief advantage in early life

Table 1. Summary of Arctic char eggs hatched from 12/1996 to 5/2001. Numbers in parentheses are numbers of accumulated thermal units (ATU) in °C (ATU = Σ [culture temperature (°C) x days at culture temperature]). For temperature exposure, the first number is incubation and hatching temperature $\pm 0.5^\circ\text{C}$, the second number is temperature at transfer from incubator to fry tanks $\pm 0.5^\circ\text{C}$. The third number is temperature at 630 ATU $\pm 0.5^\circ\text{C}$. Lab = Labrador strain, Nau = Nauyuk strain, Hybrid = Nauyuk/Tree Rivers; 2n = Diploid, 3n = Triploid. From 12/20/96 to 12/9/98, eggs were hatched at the USGS National Fish Health Laboratory, Leetown, WV. From 5/21/99 to end of the project, they were hatched at the Freshwater Institute.

Date (ATU post-fertilization) shipment received	Temperature exposure (°C) ¹	No. eggs	Date (ATU pre-hatch) 50% hatched	Date (ATU post-hatch) moved to tank	% survival to hatch	% survival to 1000 ATU
12/20/96 (347.6)	6.5-6.5-11.5 Lab (2n)	9000	1/3/97 (435.5)	1/24/97 (136.1)	96	88
12/20/96 (347.6)	8.5-8.5-11.5 Lab (2n)	18000	1/3/97 (436.0)	1/15/97 (92.6)	96	85
12/20/96 (347.6)	9.5-9.5-11.5 Lab (2n)	18000	12/31/96 (438.1)	1/9/97 (91.0)	96	77
12/2/97 (322)	6.5-6.5-11.5 Nau (2n)	3,300	12/22/97 (462)	1/14/98 (158)	98	95
12/2/97 (322)	6.5-11.5-11.5 Nau (2n)	4,100	12/22/97 (462)	1/14/98 (166)	99	97
11/18/97 (340)	11.5-11.5-11.5 Nau (2n)	6,500	11/29/97 (445)	12/11/97 (156)	99	67
12/18/97 (326)	6.5-6.5-11.5 Lab (2n)	5,100	1/13/98 (503)	2/2/98 (137)	94	79
12/18/97 (326)	6.5-11.5-11.5 Lab (2n)	5,300	1/13/98 (503)	2/2/98 (141)	93	86
12/18/97 (326)	11.5-11.5-11.5 Lab (2n)	5,300	1/2/98 (469)	1/11/98 (121)	90	74
11/24/98 (351.6)	8.5 ³ -12-12 Nau (3n)	5000	12/12/98 (485.05)	12/30/98 (170.6)	99	80
11/24/98 (356.6)	8.5-12-12 Nau (2n)	5000	12/11/98 (480.85)	12/30/98 (182.0)	99	64
12/9/98 (N/A)	8.5 ³ -12-12 Lab (2n)	5000	1/7/99 (N/A)	1/26/99 (193.95)	79	76
5/21/99 (379.5)	6.0-14.0-14.0 Nau (2n)	22500	6/3/99 (459.6)	6/26/99 (160)	93	87
5/21/99 (379.5)	6.0-14.0-14.0 Nau (2n)	13200	6/10/99 (483.0)	6/26/99 (118.2)	98	86
5/21/99 (357.5)	6.0-14.0-14.0 Nau (3n)	5000	6/10/99 (479.0)	6/26/99 (118.1)	98	80
11/18/99 (376.1)	6.0-12.0-12.0 Nau (2n)	5250	12/10/99 (502.9)	12/30/99 (143.3)	99	95
11/18/99 (354.1)	6.0-12.0-12.0 Hybrid (3n)	5250	12/10/99 (480.9)	12/30/99 (143.3)	100	89
11/18/99 (381.1)	6.0-12.0-12.0 Nau (3n)	5250	12/9/99 (502.2)	12/30/99 (149.0)	99	88
11/18/99 (376.1)	6.0-12.5-12.5 Nau (2n)	6400	12/10/99 (503.0)	1/2/00 (175.6)	99	84
11/18/99 (359.1)	6.0-12.5 ² -12.5 Nau (2n)	56100	12/12/99 (498.3)	1/3/00 (177.1)	99	93
5/25/00 (362.4)	6.1-12.3-13.2 Nau (2n)	42000	6/15/00 (492.9)	7/8/00 (158.1)	98	92

¹All Labrador strain eggs were from Brookvalley Marine Farms, Atlantic Sea Smolt Ltd., PEI, Canada. All Nauyuk strain eggs were from Icy Waters International, Inc., Yukon, Canada.

history metabolism that provides a differentiation wedge that creates much long term heterogeneity.

D. Growth in recirculating system

- Since 1999, Arctic char have been cultured in Freshwater Institute's research recirculating system. These fish were hatched at 6°C in a chilled recirculating system. When they were ready to feed, they were transferred to fry tanks in flow-through conditions, then to a partial reuse system when they were about 15-20 gms. At about 150 to 200 gms they were transferred to a recirculating system with about one to two complete turnovers per day. Average water quality conditions were: Temperature = 13-14°C; Dissolved Oxygen = 9.0-12.0 ppm; Carbon Dioxide = 17-18 ppm; pH = 7.5-7.6; Alkalinity = 250 ppm; Total Ammonia Nitrogen = 0.3-0.5 ppm; NH₃ = 0.003. Fish were fed by automatic feeders a high energy commercial salmon or trout growers diet of between 42 and 46% protein and 16 to 19% fat (e.g., Moore-Clark 47:19; Ziegler 46:18 or Silver Cup 45:19). From December, 1999 to mid-2001, growth of three cohorts of char were compared, Nauyuk¹ strain char hatched in December, 1999, Nauyuk strain char hatched in June, 2000 and Nauyuk/Tree Rivers strain char hatched in December, 2000 (Fig. 3). Growth of the cohort of hybrid strain fish has been faster than either of the two cohorts of pure strain Nauyuk char. For example, hybrid strain fish reached an average weight of 500 grams by 350 d post-hatch, where pure strain Nauyuk char reached 500 grams at about 400 d post-hatch.

¹ All Nauyuk strain fish were Icy Waters Yukon Gold char.

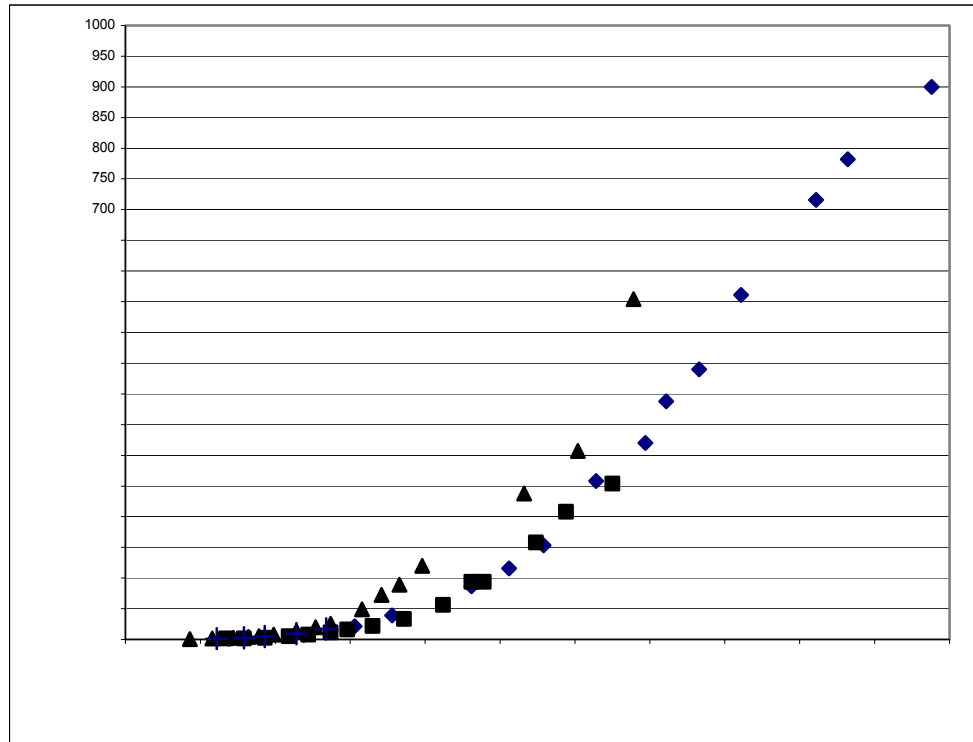


Fig. 3. Growth (average weight in grams) of some char cohorts cultured at the Freshwater Institute. (Nauyuk char hatched in June, 2000, ◆; Nauyuk char hatched in December, 1999, ■; Nauyuk/Tree Rivers Hybrid hatched in December, 2000, ▲)

V. Fish health

A. Studies of viral pathogens

1. Susceptibility

- McAllister, P.E., J. Bebak and B.A. Wagner. 2000. Susceptibility of Arctic char to experimental challenge with Infectious Hematopoietic Necrosis Virus (IHNV) and Infectious Pancreatic Necrosis Virus (IPNV). *Journal of Aquatic Animal Health* 12:35-43.** Arctic char *Salvelinus alpinus* were exposed to the fish pathogens infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) to estimate susceptibility of the species to infection. Fish were immersion challenged at 46, 74, and 95 d posthatch for 5 h in viral concentration of 10^3 or 10^5 PFU (plaque-forming units)/mL and were monitored for 21 d postchallenge. Fish that died were assayed individually for virus-associated mortality, and survivors were assayed individually to estimate prevalence of virus carrier status. Arctic char succumbed to IHNV infection with population losses as high as 41%, and prevalence of IHNV carriers in surviving fish was 24% or less with virus titers in carrier fish ranging from 2.5×10^2 PFU/g to greater than 1.3×10^6 PFU/g. Infections with IPNV resulted in losses of 20%

or less, and prevalence of IPNV carriers in surviving fish was as high as 82% percent with virus titers in carrier fish ranging from 2.5×10^6 PFU/g to greater than 5.0×10^6 PFU/g. Logistic regression models were developed for the mortality and infection data for each virus. For IHNV, the odds of dying or becoming infected were significantly greater for the fish from the 74-d age-group than for the fish from the 46-d or 95-d age-groups. Fish exposed to 10^5 PFU of IHNV/mL had significantly greater odds of dying or becoming infected than fish exposed to 10^3 PFU/mL. For IPNV, the odds of dying were significantly greater for the 74-d age-group compared with the other two age-groups. The 74-d fish were also significantly more likely to become infected than the 95-d-old fish. Fish exposed to 10^5 PFU of IPNV/mL had significantly greater odds of dying or becoming infected than fish exposed to 10^3 PFU of IPNV/mL.

2. Epidemiology

- **McAllister, P.E. and J. Bebak. 1997. Infectious pancreatic necrosis virus in the environment: relationship to effluent from aquaculture facilities. *Journal of Fish Diseases* 20:201-207.** Effluents from three fish hatcheries were monitored for the discharge and subsequent downstream distribution of infectious pancreatic necrosis virus (IPNV). Samples of spring water and surface water, and tissues from salmonid and non-salmonid fish were assayed for IPNV. Water samples were processed to recover virus by adsorption to an electropositive, micro-porous filter matrix. No IPNV was detected in surface water collected above fish hatcheries or in hatchery spring water supplies. The virus could be detected for at least 19.3 km below the point of effluent discharge from hatcheries and the prevalence of IPNV infection in stream-resident fish was 2.8%.
- **Bebak, J., P.E. McAllister and G. Smith. 1998. Infectious pancreatic necrosis virus: Transmission from infectious to susceptible rainbow trout fry. *Journal of Aquatic Animal Health* 10:287-293.** Fry of rainbow trout, *Oncorhynchus mykiss*, were exposed to serotype VR-299 of infectious pancreatic necrosis virus (IPNV) by using a standardized immersion challenge. In concurrent experiments, fish were monitored for 11 d for excretion of IPNV or monitored for 9 d for excretion and transmission of IPNV to susceptible rainbow trout fry. Immersion-challenged fish began excreting virus within 2 d after challenge. The rate of IPNV excretion per fish increased steadily from about day 4 to day 8 and then decreased. Virus concentrations in tissues of immersion-challenged fish increased exponentially. Susceptible fish became infected with IPNV within 4 d after being introduced to immersion-challenged fish (i.e., 2 d after the challenged fish began excreting virus). By 9 d, 84% of the susceptible fish were infected with IPNV.
- **McAllister, Philip E. and Julie Bebak. 1999. Effects of chronic low-level exposure to infectious pancreatic necrosis virus (IPNV) on early life stages of rainbow trout, *Oncorhynchus mykiss*. In: 24th Annual Eastern Fish Health Workshop (R. Cipriano, ed.), March 9-11, Atlantic Beach, NC.** In a previous study, we documented that infectious pancreatic necrosis virus (IPNV) was

detected in stream water for a distance of at least 19.3 kilometers below the point of effluent discharge from a fish hatchery. A low prevalence of IPNV infection was found in stream resident fishes. However, notable IPNV titer (3.0×10^4 plaque forming units (PFU)/gram of tissue), but no clinical signs of disease, was detected in a young trout--ostensibly the progeny of in-stream reproduction. In the current study, we conducted laboratory trials to estimate the effects of chronic, low-level IPNV exposure on early life stages of rainbow trout, *Oncorhynchus mykiss*. Using three ambient concentrations of IPNV (10^1 , 10^3 , and 10^5 PFU/liter of water) to simulate conditions found in the affected stream, eyed eggs of rainbow trout were exposed continuously to IPNV beginning 7 days before hatch, and exposure was continued through alevin, fry, and fingerling life stages until progeny were 44 days old. Cumulative IPNV-related mortality and IPNV-carrier prevalence in survivors were affected by ambient virus concentration and by population density. At low population density (about 35 fish/one liter tank; water flow = 250 milliliters/minute), IPNV-related mortality began at 19 days post hatch. As ambient virus concentration increased, average cumulative IPNV-related mortality increasing from 0% to 22%, and average IPNV-carrier prevalence in survivors increasing from 0% to 100%. At high population density (about 140 fish/one liter tank; water flow = 250 milliliters/minute), IPNV infection was detected at 6 days post hatch, and IPNV-related mortality began at 16 days post hatch. As ambient virus concentration increased, average cumulative IPNV-related mortality increased from 1% to 95% and average IPNV-carrier prevalence in survivors increased from 0% to 68%. Ambient virus concentration, population density, and excretion augmentation of ambient virus interacted to pressure threshold conditions requisite to initiate and sustain infection and disease.

- **Bebak-Williams, P.E. McAllister, R. Boston, G. Smith. (Co-author reviews received, revision in progress). The effect of fish density and number of infectious fish on the survival of rainbow trout fry during epidemics of infectious pancreatic necrosis. *Journal of Fish Diseases*.** Two cohort studies were carried out to compare the effect of fish density and number of infectious fish on characteristics of survival of rainbow trout fry during epidemics of infectious pancreatic necrosis (IPN). Survival analysis was used to determine whether time to death, the peak hazard rate, the time at which the peak hazard rate occurred and the rate at which the peak hazard rate was approached and departed were associated with fish density and number of infectious fish added. Loglogistic regression analysis of the survival data revealed that fish density, number of infectious fish (ie., pathogen concentration) and the interaction between the two variables significantly affected time to death from IPN ($p < 0.01$). Deterministic modeling of the hazard functions demonstrated that when pathogen concentration was low, as density increased the peak death rate increased, the time of the peak death rate decreased and the probability of survival to the end of the experiment decreased. When pathogen concentration was high, the effect of density was weaker and the peak death rate, time of peak death rate and probability of survival to the end of the experiment were more similar.

B. Studies of bacterial pathogens

1. Susceptibility

- **Clifford E. Starliper, Pete Bullock, Julie Bebak-Williams, Bradley Yeakle, Amy Spurgeon. Susceptibility of two strains of Arctic char to experimental challenge with selected salmonid bacterial pathogens. (Manuscript in preparation)** In recent years, aquaculturists have increasingly begun to intensively rear Arctic char in regions such as the mid-Atlantic U.S., which have warmer water temperatures. Currently, relatively little is known of the susceptibility of Arctic char to recognized primary bacterial pathogens of salmonids. However, diseases are likely to become more prevalent with increased intensive culture. This study was done to evaluate and compare pathogen susceptibilities of Nauyuk and Labrador Arctic char (*Salvelinus alpinus*) to each other, and to rainbow (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Groups of fingerlings were artificially exposed by intraperitoneal (IP) and intramuscular (IM) injections and waterborne (bath) exposure. Observation periods for development of disease and mortality followed challenge. The causal bacterium was recovered from dead fish and identified to confirm Koch's Postulates. Bacterial isolates were characterized using standard biochemical methods and media. For the injection challenges, bacteria were grown in an appropriate medium, then ten-fold dilutions were prepared and 0.1 mL of each dilution was injected per fish. For the waterborne challenge, bacteria were grown and fish were exposed to both non-diluted and 10^{-1} concentrations for between 1-5 min, depending on the pathogen. Clean-stock and infected fish were maintained in flow through tanks fed with 12°C pathogen-free spring water at about 1.5 L per minute. To summarize, with all pathogens evaluated, the two Arctic char strains were similar and were either the most sensitive host or they were comparable with the most sensitive. Six *Yersinia ruckeri* isolates were utilized, three were of the biotype I (sorbitol fermentation negative) and three were biotype II (sorbitol positive). Both char strains were highly susceptible to both biotypes by injection and bath exposure. Rainbow trout were similar to Arctic char while brook trout were relatively far more resistant. Host responses were similar for challenges with *Renibacterium salmoninarum* and *Aeromonas salmonicida*. Nauyuk and Labrador char were highly susceptible to both pathogens and were similar to brook trout while rainbow trout were very resistant.
- **Bacterial pathogen transmission studies between the freshwater bivalve *Amblema plicata* and Arctic char (*Salvelinus alpinus*). Clifford E. Starliper, Jay Mathias, Jennifer Hickerson, Erin McKee.** Freshwater bivalves are very susceptible to environmental and biological factors. Dams, construction, siltation and most recently the unwanted introduction of zebra mussels (*Dreissena polymorpha*) have resulted in greater than 70% of the animals native to North America to become listed as threatened, endangered or of special concern. Conservation efforts have been initiated; one is a relocation program whereby animals would be collected and propagated at facilities (including fish hatcheries)

free of their natural threats with the ultimate goal of reintroduction. With relocation, questions arose regarding the potential to introduce fish pathogens along with the mussels. This project was initiated in an effort to address these concerns. A method was developed for isolation of bacteria from bivalves and this was employed to explore the dynamic nature of the bivalves' flora including the response to a changing water quality. A study was done to determine if the required 30-day quarantine in pathogen-free water would also provide for the mussels to depurate fish bacterial pathogens, thereby eliminating the risk of contagion via relocation. There are no bacterial pathogens known to cause disease to both mussels and fish, therefore, a model system was developed for study utilizing the bivalve *Amblema plicata*, Arctic char (*Salvelinus alpinus*) and *Aeromonas salmonicida*. An artificial epizootic was established by injecting (IP) Arctic char with viable *A. salmonicida*. These fish were placed in a tank with non-injected char, which became infected via cohabitation. The injected fish died and after the non-injected fish began to die, *A. plicata* were placed in the tank with the char. When the *A. plicata* had become carriers of, and potential vectors for *A. salmonicida*, they were transferred to four tanks and pathogen-free Arctic char were added after 1, 5, 15 and 30-day quarantine periods. Transmission of *A. salmonicida* from the mussels to the "clean" char was determined by bacteriological culture and observation of disease and mortality. It was found that a quarantine period in pathogen-free water is effective to prevent transmission of *A. salmonicida*, but effectiveness is dependent on the length of the quarantine.

- **Starliper, C.E. 2001. A disease in Arctic char caused by *Serratia liquefaciens*. Page 5 In: 26th Annual Eastern Fish Health Workshop, Clarion Inn Hotel and Conference Center, Shepherdstown, WV.** Studies have indicated Arctic char to be an excellent candidate species for aquaculture at (warmer) water temperatures of 10-13°C, e.g. the mid-Atlantic U.S. With this increased interest it will become important to identify pathogens and ways to avoid and treat diseases. *Serratia liquefaciens* was a cause of morbidity and mortality in Nauyuk and Labrador strains of Arctic char that were being maintained at our lab. (*S. liquefaciens* has not previously been associated with epizootics in Arctic char, but epizootics have been described in Atlantic salmon and turbot) Mortality commenced at various times, in various sizes, of both strains of char, but did not exceed 2% per week in any lot. Except for redness and slight swelling around the anus, no external lesions were evident. Internal tissues were severely hemorrhagic with copious bloody ascites. *S. liquefaciens* was isolated from kidney, brain, spleen, swim bladder, intestine, liver and ascites. Isolates were Gram-negative rods, produced gas from glucose, were motile at 21 and 37°C, oxidase negative, A/AG in TSI, and reduced nitrates to nitrites. They were positive for gelatin, citrate, esculin, lysine, ornithine, DNase and grew on 1% m-tartrate and 1% D-malate. Isolates were negative for indole, H₂S, malonate, phenylalanine, and arginine. Groups of fish (34 g each) were exposed to viable *S. liquefaciens* by immersion in 3.48x10⁹ cfu/mL for 2 min; the only mortality was in Atlantic salmon (27%); dead fish displayed severe internal hemorrhaging, but no external signs. Upon IP injection, LD₅₀ values ranged 1.87x10⁸ to 1.41x10⁶ cfu per fish; the

two char strains and Atlantic salmon were similar, and more susceptible than brook and rainbow trout. IM LD₅₀ values were lower, 1.11x10⁶ to 1.50x10³ cfu per fish; Nauyuk and Labrador char were similar, and were more susceptible than Atlantic salmon, brook and rainbow trout. Additionally: 1) there was relatively minimal mortality among char and salmon injected IP and IM with cell-free supernatant (CFS; 0.03 mg/fish) 2) CFS mortality were hemorrhaged internally and some IM fish had raised areas at the site of injection 3) among IP injected char, mortality (LD₅₀'s: 4.37x10⁵, 1.85x10⁶ cfu/fish) was slightly lessened by washing cells 4) when filtered CFS was recombined with washed cells, mortality was comparable to that of unwashed cells. Isolates were sensitive to nalidixic acid, neomycin, oxolinic acid, Romet, streptomycin, and trimethoprim. They were resistant to ampicillin, colistin, nitrofurantoin, sulfadiazine, and tetracycline and intermediate to chloramphenicol, oxytetracycline.

2. Methods Development

- **Starliper, C.E., W.B. Schill and J. Mathias. 1998. Performance of serum-free broth media for growth of *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms* 34:21-26.** Growth of *Renibacterium salmoninarum* was compared in 14 different broth media, 13 serum-free, and 1 that contained newborn calf serum, KDM2+M. Supplementation with 1% v/v *R. salmoninarum* MCO4M metabolite was evaluated for 6 of the media that do not utilize it as part of their ingredients. Viable cells were enumerated on Days 10, 20, and 30 post-inoculation to evaluate performance. The experiment was repeated 3 times using high, low and medium (trials 1 to 3, respectively) cell concentrations as inoculum. In general there was no optimal medium and all performed well. The choice of which to employ depends on the ease of preparation and presence of certain ingredients that might affect subsequent assays. In trials 2 and 3, the pH was estimated using test papers at the same time as cells were counted. Maximum pH increase incurred with KDM2+M and those media containing charcoal. For most media, a simple pH determination could be used as a means to check that growth has occurred in a culture, particularly if charcoal was added directly to the media and a visual inspection could not be made to detect growth.
- **Cipriano, R. C. and G.L. Bullock. 2001. Evaluation of commercially prepared transport systems for non-lethal detection of *Aeromonas salmonicida* in Salmonid fish. *Journal of Aquatic Animal Health* 13:96-104.** In vitro studies indicated that commercially prepared transport systems containing Amies, Stuart's and Cary-Blair media worked equally well in sustaining the viability of the fish pathogen *Aeromonas salmonicida*, which causes furunculosis. The bacterium remained viable without significant increase or decrease in cell numbers for as long as 48 h of incubation at 18-20°C in Stuart's transport medium; consequently, obtaining mucus samples in such tubes were comparable to on-site detection of *A. salmonicida* by dilution plate counts on Coomassie Brilliant Blue agar. In three different assays of 100 samples of mucus from Atlantic salmon, *Salmo salar*, infected subclinically with *A. salmonicida*, dilution

counts conducted on-site proved more reliable for detecting the pathogen than obtaining the samples in the transport system. In the on-site assays, dilution counts detected the pathogen in 34, 41, and 22 samples, whereas this was accomplished in only 15, 15, and 3 of the respective samples when the transport system was used. In an additional experiment, Arctic char, *Salvelinus alpinus*, sustaining a frank epizootic of furunculosis were sampled similarly. Here, too, dilution counts were more predictive of the prevalence of *A. salmonicida* and detected the pathogen in 46 mucus samples; in comparison, only 6 samples collected by using the transport system were positive. We also observed that the transport system supported the growth of the normal mucus bacterial flora. Particularly predominant among these were motile aeromonads and *Pseudomonas fluorescens*. In studies of mixed culture growth, two representatives of both of the latter genera of bacteria outgrew *A. salmonicida*—in some cases, to the total exclusion of the pathogen itself.

3. Vaccination

- **Bebak-Williams, J., G.L. Bullock, B.A. Wagner. (In presubmission review by coauthors). Vaccination against furunculosis in Arctic char: comparison of two commercial vaccines. (Journal of Aquatic Animal Health).** Arctic char (*Salvelinus alpinus*) are susceptible to furunculosis, a serious bacterial disease that affects the productivity of salmonid farms around the world. The objective of this laboratory study was to compare the efficacy of two commercial furunculosis vaccines against furunculosis in two strains of Arctic char. Labrador and Nauyuk char were injected with Aqua Health Furogen 2, Alpharma Biojec 1500 or 0.9% NaCl. At 87 d and 108 d post-vaccination fish were challenged, by immersion, with *Aeromonas salmonicida*. For both challenges, the Aqua Health vaccine afforded more protection than the Alpharma vaccine. Percent cumulative mortality was significantly greater for the fish vaccinated with the Alpharma or 0.9% NaCl than it was for the fish injected with the Aqua Health product (p-value = 0.001). Differences between fish strain (p = 0.14), days since vaccination (p-value = 0.24) and the interaction between strain and day (p-value = 0.60) were not significant. For both challenges, relative percent survival (RPS) was greater than 90% for the Labrador fish vaccinated with the Aqua Health product and was less than 20% for the Nauyuk fish vaccinated with the Alpharma product. For the Nauyuk strain char, RPS for the Aqua Health product was 85.1% and 35.5% for 87 and 108 d, respectively. The RPS for the Nauyuk strain for the Alpharma product was less than 10% for both trials.

4. Identification of a Rickettsia-like organism (RLO)

- **Noble, A.C. 1999. Case history: Gill pathologies associated with acute and chronic mortalities in arctic char (*Salvelinus alpinus*) cultured in a semi-closed recycle system. In: 24th Eastern Fish Health Workshop (R. Cipriano, ed.), March 9-11, Atlantic Beach, NC.** A cohort of 2520 arctic char (*Salvelinus*

alpinus) with an average size of 270 gms was introduced into a semi-closed recycle system at a density of 71.6 kg/m³. About two months after introduction, fish stopped gaining weight and feed consumption decreased, followed several days later by no feeding response. Mortalities of a few fish per day occurred for about a week, then increased, peaked at about 225 fish per day and then decreased. Macroscopically, gill filaments had inflamed tips and white patches. Excess mucus and hyperplasia of the gills were observed on wet mounts. All cultures for systemic bacteria, FA tests, and wet mount observations were negative for pathogens. Assays for IPNV, IHNV, VHSV and ISAV were also negative. Feed samples were negative for rancidity and mycotoxins. Histopathology samples revealed severe chronic-active gill inflammation that was most likely responsible for the high mortalities. All fish in the sample taken during acute mortalities showed a severe chronic diffuse granulocytic and suppurative proliferative branchitis with secondary lamellar fusion and mucus cell hyperplasia. Numerous eosinophilic intranuclear inclusions with primary lamellar epithelial cells were commonly associated with the hyperplasia and inflammation in severely affected fish. These inclusions did not stain as DNA with a Fielgen's stain, and did not appear to resemble viral particles ultrastructurally. In one of the sample groups gill tissue contained irregular granular basophilic intracytoplasmic inclusions within scattered epithelial cells of secondary lamellae. These PAS-negative inclusions stained intensely acidophilic with Gram's stain but did not appear to be bacteria. The same inclusions were red when a Machiavello's stain was utilized; however their morphology and tinctorial properties were not identical to the epitheliocystis inclusions that have been observed in the gills of tilapia. Electron microscopy of the epithelial cells of the gill showed a large, single, centrally located aggregate of amorphous electron-dense granular material most consistent with nucleolar hypertrophy. Immunohistochemistry performed on gill samples using anti-chlamydial antibodies was negative.

- ***Further studies on the rickettsial-like-organism.*** Since the preceding abstract was first published, outbreaks of respiratory disease have occurred in many of the char cohorts cultured at the Freshwater Institute. Further descriptions of the agent associated with the outbreaks indicate that it is a rickettsial-like-organism (RLO), a Gram (-) intracellular coccoid agent. With cooperation from scientists at two USDA-ARS laboratories and the USGS National Fish Health research laboratory, studies to isolate, identify and culture the organism are continuing.

5. Epidemiology

- **Bebak, J., M. Baumgarten and G. Smith. 1997. Risk factors for bacterial gill disease in young rainbow trout (*Oncorhynchus mykiss*) in North America. *Preventive Veterinary Medicine* 32:23-34.** A retrospective whole-population survey was used to investigate putative risk factors for bacterial gill disease (BGD) in young hatchery-reared rainbow trout in North America. Three sets of analyses were done. The first analysis included as cases all of the hatcheries in which there was at least one outbreak of BGD during the 2-year study interval,

regardless of the location of the outbreak in the hatchery. The case group for the second analysis was limited to hatcheries for which the BGD outbreak occurred outside of the hatch house. For the logistic regression that combined all cases of BGD (regardless of location of the outbreak), there was a significant association between mortality from bacterial gill disease and previous experience with BGD outbreaks (odds ratio [OR] = 5.2; 95% confidence interval [CI] = 5.6, 18.2), being a commercial trout hatchery (OR = 5.2; 95% CI = 2.6, 10.4), and being a hatchery with an annual salmonid fish production of > 250,000 fish (OR = 2.9; 95% CI = 2.2, 12.6), as did the use of ultraviolet radiation to disinfect the hatch house water (OR = 7.5; 95% CI = 2.2, 25.8), previous experience with bacterial gill disease (OR = 19.3, 95% CI = 7.9, 46.8), and being a commercial hatchery (OR = 7.7, 95% CI = 3.2, 18.6). The odds of a BGD outbreak outside of the hatch house was significantly associated with previous experience with BGD (OR = 4.3; 95% CI = 2.2, 8.6) and with being a hatchery with an annual salmonid fish production > 50,000 pounds (OR = 2.5 95% CI = 1.2, 5.1).

6. Treatment

- **Bebak-Williams, J., G. Bullock, M.C. Carson. 2001. (In Press). Oxytetracycline residues in a freshwater recirculating system. *Aquaculture*.** When oxytetracycline (OTC) medicated feed is fed to fish in a recirculating aquaculture system, antibiotic residues could accumulate in fish tissue, water, biofilter sand and sediment to a greater extent than in single pass or serial reuse aquaculture systems. In two trials, oxytetracycline-medicated feed (3 g active ingredient per pound of feed) was fed to adult rainbow trout at 1% b.w. per day for 10 days. OTC residues were assayed in fish muscle (with skin attached), water, sediment (e.g., fish feces, uneaten feed) and biofilter sand. For both trials, oxytetracycline was detected during the 10 days of treatment in all matrices assayed. In trout muscle, OTC concentrations increased to an average of 1.8 µg/g by day 10 of treatment and then declined to <0.2 µg/g by 21 days post-treatment. For water entering and exiting the biofilter, OTC concentrations increased to 0.5 µg/mL by day 10 and was not detectable (<0.001 µg/mL) by 21 d post-treatment. For biofilter sand, OTC concentration was approximately 14 µg/g by day 10 and decreased to <2 µg/g by 21 d post-treatment. In sediment samples, OTC concentrations increased to 1900 µg/g by day 10 and declined to <2 µg/g by 21 days post-treatment. In this system, OTC concentrations in trout muscle were well below 2 µg/g by 21 d after withdrawal of the drug. After input of medicated feed to the system was stopped, OTC concentrations in water, sediment and the biofilter declined and did not increase during the post-treatment period.
- **Carson, M.C., D. Patel, G. Bullock, J. Bebak-Williams. 2001. (In press). Determination of oxytetracycline residues in matrices (trout, biofilter sand, sediment, water) from a freshwater recirculating system. *Journal of AOAC International*.** This collection of related procedures determines the amount of oxytetracycline (OTC) present in trout tissue (muscle with skin attached), biofilter sand, sediment, and tank water from a recirculating aquaculture system. OTC is

extracted from the matrices using different techniques, depending on complexity of the matrix and desired OTC detection level in that matrix. Listed in order of increasing complexity, OTC is extracted from tank water by dilution with acidic buffer containing ethylenediaminetetraacetic acid (EDTA); from biofilter sand by shaking with 0.1N HCl; from sediment by homogenization and shaking with buffer/EDTA; from ground trout by homogenization and shaking with buffer/EDTA (twice), with further cleanup and concentration of the extract on a polymeric solid-phase extraction (SPE) cartridge. The four procedures all use the same reversed-phase gradient chromatography on a polymeric column with UV detection at 350 nm. The lower limit of detection (estimated) and upper limit of validation for each of these four matrices are 0.04 to 4.0 ug/g (ppm) (trout), 0.03 to 20 ppm (biofilter sand), 1 to 6,000 ppm (sediment), and 0.003 to 10 ppm (water). Recoveries range from 82% to 108%, with RSD <20% over the applicable concentration ranges. These procedures were used to monitor OTC residues resulting from medicated feed fed to rainbow trout in a recirculating aquaculture system.

VI. Outcomes

A. Peer-reviewed publications

- Bebak, J., M. Baumgarten and G. Smith. 1997. Risk factors for bacterial gill disease in young rainbow trout (*Oncorhynchus mykiss*) in North America. *Preventive Veterinary Medicine* 32:23-34. (Abstract in Section V.B.)
- Bebak, J., J.A. Hankins and S.T. Summerfelt. 2000. The effect of water temperature on the survival of eyed eggs and alevins of Arctic char. *North American Journal of Aquaculture* 62:139-143. (Abstract in Section IV.B.)
- Bebak, J., P.E. McAllister and G. Smith. 1998. Infectious pancreatic necrosis virus: Transmission from infectious to susceptible rainbow trout fry. *Journal of Aquatic Animal Health* 10:287-293. (Abstract in Section V.A.)
- Bebak-Williams, J., G. Bullock, M.C. Carson. 2001. (*In Press*). Oxytetracycline residues in a freshwater recirculating system. *Aquaculture*. (Abstract in Section V.B.)
- Bebak-Williams, J. and G.L. Bullock. (Coauthor reviews received, revision in progress). Vaccination against furunculosis in Arctic char: comparison of two commercial vaccines. (*Journal of Aquatic Animal Health*). (Abstract in Section V.B.)
- Bebak-Williams, P.E. McAllister, R. Boston, G. Smith. (*Co-author reviews received, revision in progress*). The effect of fish density and number of infectious fish on the survival of rainbow trout fry during epidemics of infectious pancreatic necrosis. *Journal of Fish Diseases*. (Abstract in Section V.A.)

- Carson, M.C., D. Patel, G. Bullock, J. Bebak-Williams. 2001. (*In press*). Determination of oxytetracycline residues in matrices (trout, biofilter sand, sediment, water) from a freshwater recirculating system. *Journal of AOAC International*. (Abstract in Section V.B.)
- Cipriano, R. C. and G.L. Bullock. 2001. (*In Press*). Evaluation of commercially prepared transport media systems for non-lethal detection of *Aeromonas salmonicida*. *Journal of Aquatic Animal Health*.(Abstract in Section V.B.)
- Jittinandana, S., P.B. Kenney, S.D. Slider, P. Mazik, J. Bebak-Williams, and J.A. Hankins. *In presubmission review by coauthors*. Effect of handling stress on quality of smoked Arctic Char (*Salvelinus alpinus*) fillets. *J. Food Science*. Arctic char, *Salvelinus alpinus*, hatched at 6.5 and 9.5 °C and later divided into large, medium and small size groups were assigned to: 1) processing with no handling stress (control); 2) processing immediately after handling stress; 3) processing 24 h after handling stress; and 4) processing 48 h after handling stress. For the no-stress control, fish were quickly removed from the water. Handling stress was imposed by crowding fish in shallow water with a net for 5 min. Stress reduced gut weight and muscle a* value, increased muscle L* for the 9.5 °C group, decreased muscle L* for the 6.5 °C group, and increased fillet weight loss after brining. Fish hatched at 9.5 °C appeared to be more sensitive to handling stress than fish hatched at 6.5 °C. Fillets from the stressed, 9.5 °C group absorbed more brine, increasing salt and ash content of smoked products. Resting for 24 h after stress decreased total and water-phase salt, and therefore, ash content. Resting for 48 h increased gut weight and enhanced muscle L*. However, muscle a* was not recovered after a 48-h resting period.
- McAllister, P.E. and J. Bebak. 1997. Infectious pancreatic necrosis virus in the environment: relationship to effluent from aquaculture facilities. *Journal of Fish Diseases* 20:201-207. (Abstract in Section V.A.)
- McAllister, P.E., J. Bebak and B.A. Wagner. 2000. Susceptibility of Arctic char to experimental challenge with Infectious Hematopoietic Necrosis Virus (IHNV) and Infectious Pancreatic Necrosis Virus (IPNV). *Journal of Aquatic Animal Health* 12:35-43. (Abstract in Section V.A.)
- Starliper, C.E., W.B. Schill and J. Mathias. 1998. Performance of serum-free broth media for growth of *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms* 34:21-26. (Abstract in Section V.B.)
- Starliper, C.E. 2001. The effect of depuration on transmission of *Aeromonas salmonicida* between the freshwater bivalve *Amblema plicata* and Arctic char. *Journal of Aquatic Animal Health*. 13:56-62. A model system was used to study bacterial fish pathogen transmission between the freshwater bivalve, *Amblema plicata*, and two strains (Nauyuk and Labrador) of Arctic char, *Salvelinus alpinus*. *Aeromonas salmonicida*, the cause of fish furunculosis, was readily transmitted from Arctic

char to *A. plicata* and vice versa via simple cohabitation. Clinical furunculosis was artificially established in Nauyuk Arctic char via horizontal exposure to Labrador Arctic char that received intraperitoneal injections of *A. salmonicida*. After the Nauyuk Arctic char began to die, *A. plicata* were placed in the tank with the fish. After 33 d of cohabitation, a group of 10 *A. plicata* was cultured, and *A. salmonicida* was isolated from all 10. The remaining *A. plicata* were transferred to other tanks being supplied with specific-pathogen free water. At 1, 5, 15, and 30 d after transfer, 60 uninfected Labrador Arctic char were cohabitated with the *A. plicata*. Transmission of *A. salmonicida* from *A. plicata* to the Arctic char was evaluated via fish mortality and bacterial culture after 3-4 weeks of exposure. Mortality to *A. salmonicida* occurred in groups exposed to *A. plicata* after 1 and 5 d of depuration but not in groups exposed after 15 and 30 d. The bacterium was not isolated from either the *A. plicata* or the Arctic char in the 15- and 30-d groups. Results indicate that the current minimum 30-d quarantine of freshwater bivalves destined for relocation to prevent spread of zebra mussels, *Dreissena polymorpha*, is sufficient to allow depuration of a fish pathogen and, thus, to prevent the spread of disease.

Starliper, C. E. 2001. Isolation of *Serratia liquefaciens* as a pathogen of Arctic char (*Salvelinus alpinus* L). *Journal of Fish Diseases* 24:53-56. (no abstract)

Starliper, C.E. and P. Morrison. 2000. Bacterial pathogen contagion studies among freshwater bivalves and salmonid fishes. *Journal of Shellfish Research* 19(1): 251-258. A part of the conservation efforts of native freshwater bivalves is a relocation program whereby animals are collected and moved to a safe refuge for maintenance and propagation. With the rearing of two different hosts, mussels and fish, on the same facility there is a question of the possibility for contagion of pathogens. The studies presented here are part of a continuing effort to address the concerns of contagion. Freshwater bivalves collected throughout the 1997 season were cultured for fish pathogens. Counts of total bacteria on cytophaga medium ranged between 1.07×10^5 cfu/g and 4.99×10^5 cfu/g of mussel soft tissues. The predominant groups of bacteria were motile *Aeromonas* spp. and *Pseudomonas* spp., both of which include members that are opportunistic pathogens to salmonid fishes. No primary fish pathogens were cultured; however, cells with correct morphology for *Renibacterium salmoninarum*, cause of bacterial kidney disease, were detected from mussel soft tissues in all six trials using the direct fluorescent antibody test. Groups of mussels were subjected to 24-h waterborne challenges using bacteria cultured from healthy fish; no mortality occurred to any of the animals. Another group exposed to the fish pathogen, *Aeromonas salmonicida*, also showed no effects of the challenge; however, susceptible fish became infected and died after the fish were added to cohabit with this group of mussels.

Starliper, C.E., R. Vilella, P. Morrison and J. Mathias. 1998. Studies on the bacterial flora of native freshwater bivalves from the Ohio River. *Biomedical Letters*. 58, 85-95. In response to interest in the culture of freshwater bivalves on refugia with salmonid fish, studies were initiated to examine the potential for bacterial

pathogen contagion between the two hosts. Freshwater bivalves were collected from the Ohio River in November 1995 and June and September 1996. In all, 190 animals were sampled, some from their native Ohio River water (day 0) and others after relocation to a different water supply. Sampling and bacterial isolation techniques are described. The mean total bacteria counts were about 1.6×10^5 CFU/g of bivalve soft tissue, and this remained relatively stable during the 30-day period, yet the distribution was dynamic. *Flavobacterium columnare*, a salmonid fish pathogen, was isolated from one *Amblema plicata* of a day 0 sample in June 1996 but not after relocation to a different water source.

B. Presentations with published proceedings

- **Bebak, J. 1998. The importance of biosecurity in intensive culture. Pages 245-252 in: The Second International Conference on Recirculating Aquaculture (G.S. Libey and M.B. Timmons, eds.), Virginia Polytechnic and State University, July 16-19, Roanoke, VA.** The explosive growth of worldwide aquaculture has resulted from culture intensification and from an increased number of species being cultured in an increased number of locations. As culture intensification has proceeded, catastrophic loss from infectious disease outbreaks has been repeatedly identified as a major cost to industry productivity. Major causes of disease-related financial loss are direct losses, market losses and costs resulting from lost opportunity. Direct losses include mortality, facility closure orders, restriction of movement orders, and the inability to replace stock. Market losses include reduced quality of survivors (e.g., from reduced growth rates and lower yields or reduced product quality), a restricted market for healthy stock because of damage to a facility's reputation, and missed markets. Examples of opportunity costs are diversion of management and labor and underutilization of the fish production facility (Paterson et al., 1991). The purpose of this paper is to describe: 1) how increased intensification results in an increased risk of infectious disease outbreaks; 2) summarize the major risk factors for infectious disease outbreaks in finfish culture; and 3) describe ways to use the principles of biosecurity to decrease the risk that outbreaks of infectious disease will occur at facilities where intensive culture of finfish is an economic necessity.
- **Bebak, J., P. McAllister, R. Boston and G. Smith. 2000. The role of fish density in infectious diseases outbreaks. Pages 357-360 In: Third International Conference on Recirculating Aquaculture, Virginia Polytechnic Institute and State University, July 22-23, Roanoke, VA. (no abstract)**
- **Bullock, G.L., W.B. Schill and E. Burchard. 2000. Transmission of furunculosis and enteric redmouth by means of fluidized-sand biofilters. Pages 348-352 In: The Third International Conference on Recirculating Aquaculture, Virginia Polytechnic Institute and State University, July 22-23, Roanoke, VA.** Two bacterial pathogens of salmonids, *Aeromonas salmonicida*,

cause of furunculosis, or *Yersinia ruckeri*, cause of enteric red mouth, were added for five days to the pump inlet of a recirculating culture system, without fish, to determine if the pathogens would become established in the fluidized sand biofilters and infect newly added salmonids. After culture addition, the recycle system, except for the biofilters, was disinfected with 200 ppm chlorine for two hours, drained and refilled with spring water. Biofilters were continuously washed for 24 hours with spring water to remove the pathogens, but the two pathogens could be cultured after biofilter washing. The recycle system was then stocked with rainbow trout (*Oncorhynchus mykiss*, Walbaum) to detect *Y. ruckeri* or Arctic char (*Salvelinus alpinus*, Linnaeus) to detect *A. salmonicida*. Rainbow trout began dying from enteric redmouth three weeks after being stocked, while char started dying from furunculosis within two weeks after stocking. Rainbow trout or char were then removed from the system and the entire recycle system treated with 10 ppm chloramines-T to attempt removal of the pathogen. The system was restocked with trout or char and observed for six weeks. Enteric redmouth did not occur nor could the bacterium be cultured from trout or biofilter. However furunculosis occurred in char within six weeks. In a second transmission procedure the entire recycle system was disinfected with 10 ppm chloramines-T immediately after addition of *A. salmonicida* or *Y. ruckeri* and trout or char added to the system immediately after disinfection. Six weeks after adding fish neither furunculosis or enteric redmouth occurred. *Yersinia ruckeri* could not be cultured from biofilters or trout, but *A. salmonicida* was cultured from one biofilter.

C. Presentations without proceedings

- **Bebak, J., J.A. Hankins and S.T. Summerfelt. 1998. Effects of water temperature on survival of the eggs and fry of two strains of arctic char (*Salvelinus alpinus*) Northeast Aquaculture Conference & Expo, Maine Aquaculture Innovation Center, November 18-19, Rockport, ME.**
Groundwater temperatures in the Appalachian region of the eastern United States average about 10-13°C. This water temperature has been viewed as a constraint to culture of arctic char in the Appalachian region because, according to current thinking, successful hatch and early survival of char require water temperatures of about 6 to 8°C. As part of a five-year project funded by the USDA-ARS to investigate the potential of arctic char for culture in the Appalachian region, we decided to address this constraint regarding temperature effects on hatching success and fry survival. The purpose of this study was to describe the hatching success and post-hatch survival for two strains of arctic char (labrador and nauyuk) when they were incubated and hatched at various temperature combinations between 6.0 and 12.0°C. Eyed eggs, which were obtained from two suppliers in Canada, were incubated and hatched at water temperatures ranging from 6 to 12°C. Hatching success for all temperature treatments was 90% or greater. Survival analysis was used to estimate the probability of survival to 90 days post hatch, or 1,000 accumulated thermal units (ATUs). For all groups, except those incubated, hatched and reared at 12°C, the probability of survival

was greater than 80%. At 12°C, the probability of survival varied between about 65% and 75%, depending on the strain of char.

- **Bebak, J., P.E. McAllister, R. Boston and G. Smith. 1998. The effect of fish density on the survival of rainbow trout fry during an infectious pancreatic necrosis (IPN) epidemic. In: Third International Symposium on Aquatic Animal Health, August 30-September 3, Baltimore, MD.** One consequence of the intensive culture of animals is an increased risk for outbreaks of infectious disease. Few studies have characterized or quantified this problem as it relates to the intensive culture of finfish. In controlled, replicated experiments, susceptible rainbow trout fry were exposed to infectious pancreatic necrosis virus via the introduction of fish infected with the virus. One, two or three infectious fish were introduced to tanks containing varying densities of susceptible fish. The mortality data was subjected to survival analysis, which epidemiologists often use to investigate “time to an event” problems affecting human and animal health. For susceptible fish, the probability of survival declined with fish density. Characterization of the relationship between fish density and infectious disease epidemics will facilitate the development of management strategies that optimize fish density in intensive aquaculture systems.
- **Bebak, J. P.E. McAllister and G. Smith. 1998. Infectious pancreatic necrosis virus: transmission from infectious to susceptible rainbow trout fry. In: 23rd Annual Eastern Fish Health Workshop, USGS Biological Resources Division, National Fish Health Research Laboratory, March 30-April 2, Plymouth, MA.** Rainbow trout (*Oncorhynchus mykiss*) fry were exposed to serotype VR-299 of infectious pancreatic necrosis virus (IPNV) using a standardized immersion challenge. In concurrent experiments, fish were monitored for 11 d for excretion of IPNV, or for 9 d for excretion and transmission of IPNV to susceptible rainbow trout fry. Immersion-challenged fish began excreting virus within 2 d after challenge. The rate of IPNV excretion per fish increased steadily from about day 4 to day 8, and then decreased. Virus concentrations in tissues of immersion-challenged fish increased exponentially. Susceptible fish became infected with IPNV within 4 d after being introduced to immersion-challenged fish (e.g., 2 d after the challenged fish began excreting virus). By 9 d 84% of the susceptible fish were infected with IPNV.
- **Bebak, J., P.E. McAllister, R. Boston and G. Smith. 2001. Infectious disease outbreaks: Fish density and pathogen concentration. Page 48 In: Book of Abstracts—Aquaculture 2001. World Aquaculture Society January 21-25, Lake Buena Vista, FL.** Infectious disease outbreaks in aquaculture impose a significant cost to productivity. The relationship between fish density, pathogen concentration and characteristics of infectious disease outbreaks has not been studied. A disease model was used to investigate the following questions: 1) As fish density and pathogen concentration increase, how does the probability of survival for an individual fish change? 2) How does the value for the maximum death rate change as pathogen load changes? 3) As pathogen load changes, how

does this change the time at which the maximum death rate occurs? The salmonid viral disease, infectious pancreatic necrosis (IPN), was used as the disease model. Rainbow trout (34 days old) were used for the study, which consisted of two experiments (59 d and 53 d long). Either 1, 2 or 3 fish infected with IPNV (INF fish) were added to tanks containing various densities of uninfected, susceptible fish. Additional tanks contained no INF fish or all INF fish. Tank densities ranged from about 0.05 to 0.34 lb/gal (6.0 to 40 kg/m³). The probability of survival to the end of the experiment was >0.98 for tanks where no INF fish were added. As the number of INF fish added to the tanks increased, the probability of survival to the end of the experiment decreases from 0.35 to 0.09. When each group of infectious fish is considered separately, the effect of density is apparent. For the tanks where INF = 1 or 2, the probability of survival is higher at densities <0.11 lb/gal (13.2 kg/m³) than it is at densities ≥0.11 lb/gal (13.2 kg/m³). As the number of INF fish increases, the advantage of lower densities disappears until the probability of survival at given time point is about the same regardless of the culture density. This interaction between total density and number of INF fish has been confirmed with regression models. For tanks to which one infectious fish was added, the maximum death rate was 5.2 %/d. As the number of infectious fish increased, the maximum death rate increased, until it reached 14.9 %/d in tanks where all fish were challenged by the IPN virus. When one infectious fish was added to tanks of susceptible fish, the time taken to reach the maximum death rate was 26 days. As the number of infectious fish added increased, the time to the maximum death rate decreased until it reached 13 d for tanks where all fish were infectious. Survival during an infectious disease outbreak depends on both fish density and pathogen concentration. Implementation of effective biosecurity measures must be regarded as a critical component of fish health management strategies for the intensive culture environment.

- **Bebak, J., G.L. Bullock, J.A. Hankins and S. T. Summerfelt. 1999. Effects of water temperature and vaccination on survival of two strains of Arctic char. In: Aquaculture Canada '99, October 26-29, Victoria, BC, Canada.** The Freshwater Institute is in the middle of a five-year project to identify and solve biological and technological constraints to Arctic char culture in the Appalachian region of the eastern United States, where groundwater temperatures average 10-13°C. Two studies that have been completed are: 1) the effect of early rearing temperature on hatching success and fry survival; and 2) the effect of furunculosis vaccination on survival of char after challenge with *Aeromonas salmonicida*. In the first study, eyed eggs (Labrador and Nauyuk strain) were incubated and hatched at temperatures ranging from 6 to 12°C. Hatching success for all temperature treatments was 90% or greater. For all groups, except those incubated, hatched and reared at 12°C, the probability of post-hatch survival was greater than 80%. At 12°C, the probability of post-hatch survival varied between about 65% and 75%, depending on the strain of char. In the second study, the relative percent survival after challenge with *A. salmonicida* was compared in two strains of char that were vaccinated with sterile saline or with one of two commercially available injectable furunculosis vaccines (AquaHealth and

ALPharma). For both strains of char, the AquaHealth vaccine was more protective against mortality from clinical furunculosis than the ALPharma vaccine.

- **Bebak, J., P.E. McAllister, R. Boston, and G. Smith. 1999. The effect of fish density on the survival of rainbow trout fry during an infectious pancreatic necrosis (IPN) epidemic. In: 24th Annual Eastern Fish Health Workshop (R. Cipriano, ed.), March 9-11, Atlantic Beach, NC.** One consequence of the intensive culture of animals is an increased risk for outbreaks of infectious disease. Few studies have characterized or quantified this problem as it relates to the intensive culture of finfish. In two separate controlled, replicated experiments, susceptible rainbow trout fry were exposed to infectious pancreatic necrosis virus via the introduction of fish infected with the virus. One, two or three infectious fish were introduced to tanks containing varying densities of susceptible fish. The mortality data was subjected to survival analysis, which epidemiologists often use to investigate “time to an event” problems affecting human and animal health. For susceptible fish, the probability of survival to the end of the experiment was related to fish density and the number of infectious fish that were added. For example, when one infectious fish was added to the tanks, the probability of surviving to the end of the experiment was greater for fish from the lower density tanks. However, when three infectious fish were added, the probability of survival was similar for all tanks. Characterization of the relationship between fish density and infectious disease epidemics will facilitate the development of management strategies that optimize fish density in intensive aquaculture systems.
- **Bullock, G.L. 1998. Historical perspectives of fish health research in the United States – the Leetown experience. Keynote address for the U.S. at First Russia-USA Symposium: Aquaculture and Fish Health, July 12-19, Rybnoe, Dmitrov Region, Moscow Province, Russia.** Fish health research in the United States, unlike many European and Asian countries, did not develop as a result of aquacultural activities, but rather as a consequence of raising fish species to enhance particular fisheries, such as salmonids and warmwater species. The expertise on diagnostics and research on new diseases and other areas of fish health arose within federal and state conservation agencies and a few universities. Similar to human and veterinary medicine, early research in fish health was devoted to parasites and then progressed to bacterial, viral, nutritional and environmental diseases. Early pioneers in fish health research in the U.S. include Emmeline Moore, H.S. Davis, Richard Weissenberg, Stanislaw Snieszko, and Ross Nigrelli. One of the earliest fish health laboratories established in the early 1930s by the Federal government is located in Leetown, West Virginia, about 100 kilometers from Washington, D.C. Dr. H.S. Davis supervised the research shortly after construction of the Leetown facility and became a leading authority in fish health. He was a pioneer in describing many new diseases of fish and his 1934 publication *Care and Diseases of Trout*, and later, *Culture and Diseases of Game Fish*, became standards on fish health. In 1948 Dr. S.F. Snieszko became laboratory director at Leetown, and through his vision and planning, the

laboratory gained an international reputation in many areas of fish health research and teaching. Dr. Snieszko and Dr. James Gutsell, another well known researcher, found that sulfonamides were effective in controlling bacterial diseases and established treatment regimens that are still used today. Dr. Phil Griffin provided information on new bacteriological diseases, and Dr. Edward Wood researched histopathological changes associated with bacterial and nutritional diseases. In the 1950s Dr. Snieszko hired Ken Wolf and Glenn Hoffman. Dr. Wolf established the first continuously growing cell line from trout, isolated the first viral pathogen from trout, described the life cycle of *Myxobolus cerebralis* and wrote a definitive text on fish viruses and viral diseases. Dr. Hoffman described many new species of fish parasites, developed control procedures for parasites, and wrote a definitive text on fish parasites, a revision of which will be published this year. Over the years, new researchers have been added, and the laboratory has continued to be a leader in fish health research. In addition to research, the Leetown facility provided both short-term and intensive training in diagnosis and control of fish diseases. From 1952 until 1989, a total of 419 individuals received fish health training. While the Leetown facility has had a long history of research and teaching, there are many such facilities functioning today and interest in fish health research continues to grow.

- **Bullock, G.L. 1998. Present status of three important gram negative bacterial diseases of cultured freshwater salmonids. In: First Russia-USA Symposium: Aquaculture and Fish Health, July 12-19, Rybnoe, Dmitrov Region, Moscow Province, Russia.** Bacterial diseases cause serious mortalities among several species of cultured freshwater salmonids. The most serious diseases are furunculosis, caused by *Aeromonas salmonicida*, Enteric redmouth, caused by *Yersinia ruckeri*, bacterial gill disease caused by *Flavobacterium branchiophilum*, bacterial kidney diseases caused by *Renibacterium salmoninarum*, and coldwater disease caused by *F. psychrophilum*. The present status of the first three diseases will be discussed in this presentation. Furunculosis affects most of the cultured salmonids with rainbow trout (*Oncorhynchus mykiss*) being the most resistant. The furuncle (a large external closed lesion) for which the disease was named is rarely seen except in the chronic disease state. External hemorrhages around fins and internal hemorrhages in the viscera are the most common clinical signs. Presumptive diagnosis is made by inoculation of tissue from kidney or other organs onto Coomassie Brilliant Blue agar (CBB) and observing formation of dark blue colonies in 24-48 h. A definitive diagnosis is then made by performing biochemical tests on the blue colonies. Commercial reagents are available for diagnosis by direct FAT and ELISA. Inoculating mucus or gill tissues onto CBB agar is useful in detecting latent carriers of furunculosis. Treatment of outbreaks is limited to those antibacterial approved by the U.S. Food and Drug Administration (FDA) and include oxytetracycline and the potentiated sulfonamide Romet. Enteric redmouth is principally a disease of rainbow trout and is most common in the western and southeastern United States where this species is cultured. Hemorrhages around the mouth and gill areas are common in infected trout. Several serotypes are described for *Y. ruckeri* and diagnosis is made by isolation and identification of the causative bacterium or by FAT. Oxy-

tetracycline effectively treats redmouth outbreaks but this antibacterial is not approved by the FDA. Bacterial gill disease (BGD) affects virtually all cultured salmonids and is particularly virulent for juveniles. Although other bacteria may be involved, *F. psychrophilum* is the most commonly seen bacterium in BGD outbreaks. Affected salmonids typically stop feeding and tend to line up against the current to force water over the gills. Diagnosis is made by observing hyperplastic gill lamellae containing masses of long thin Gram negative bacteria. An indirect FAT applied to gill tissue is also used for diagnosis. Large losses can occur with BGD so it is important to treat fish in the early stage of an outbreak. Quaternary ammonium compounds applied at 1-2 ppm as a bath treatment and chloramine-T as a 9-12 ppm bath are effective in controlling BGD but neither have been approved by the FDA for this purpose.

- **Bullock, G.L. and C. Starliper. 1998. Current status of bacterial gill disease in cultured salmonids. In: First Russia-USA Symposium: Aquaculture and Fish Health, July 12-19, Rybnoe, Dmitrov Region, Moscow Province, Russia.** Since the first reported outbreak of bacterial gill disease (BGD) in brook trout (*Salvelinus fontinalis*) in a hatchery in Vermont (U.S.A.) hundreds of thousands of fingerling and yearling salmonids have died from disease outbreaks. The occurrence of BGD is virtually limited to cultured salmonids and occurs worldwide. Long thin Gram negative bacteria are present on gills of affected salmonids and until the later 1970s the causative agent was considered to be one or more species of yellow pigmented soil bacteria, termed mycobacteria. However, Japanese investigators demonstrated that a yellow pigmented *Flavobacterium* was the main cause of BGD and the organism is presently named *F. branchiophilum*. The principal means of disease transmission is fish to fish and experimental transmission of BGD has been accomplished with broth cultures of *F. branchiophilum* and this organism has been identified from several disease outbreaks by indirect ELISA. The onset of BGD is characterized by fish going off feed and lining up against the current to force water over the gills. Diagnosis is accomplished by observing hyperplastic gill lamellae, which contain large numbers of flavobacteria. The mechanism of pathogenesis is not known but both pili and extracellular products have been demonstrated with *F. branchiophilum*. The pili allow bacteria to attach to gill tissue and cell free extracellular products have been shown to cause hyperplasia of gill lamellae. The cultural conditions that allow *F. branchiophilum* to colonize gill tissue and produce pathology are not understood so that prevention of BGD is not possible and treatment is the only option to limit mortality. Several chemicals applied as a bath or constant flow treatment are effective in controlling outbreaks. These include quaternary ammonium compounds at 1-2 ppm, chloramine-T at 12-15 ppm, and hydrogen peroxide at 100-200 ppm. However, in the United States none of these chemicals have been approved by the Food and Drug Administration for treating BGD. To better understand the causative agent of BGD and the epizootiology of the disease future research should include the determination of cultural conditions that predispose trout to BGD and more definitive studies on the biochemical, serological, and pathogenic characteristics of *F. branchiophilum*.

- Bullock, G.L., W.B. Schill and E. Burchard. 2000. Transmission of *Aeromonas salmonicida* and *Yersinia ruckeri* in a salmonid recycle system by means of fluidized sand biofilters. Page 5 In: 25th Annual Eastern Fish Health Workshop, John Carver Inn, April 10-14, Plymouth, MA.** Control of infectious diseases in a recirculating fish culture system can be difficult because the pathogen may become established in the biofilters and cause a reoccurrence of the disease or infect newly stocked fish. We tested whether *Aeromonas salmonicida*, causative agent of furunculosis or *Yersinia ruckeri*, causative agent of enteric redmouth disease, could become established in fluidized sand biofilters and then infect newly stocked salmonids. Each pathogen was tested separately, and after fish were removed from the recycle system, 48 hr broth cultures of the pathogen under study were continuously pumped into the system for five days. Both pathogens could be cultured from the biofilters and tank water after the five-day pumping of culture. Immediately after the addition of culture the entire system, except for the biofilters was disinfected with 200 ppm chlorine for two hours and the system was rinsed with spring water. Biofilters were rinsed for 24 hrs with spring water to attempt removal of the pathogen. After disinfecting and rinsing, the system was stocked with specific pathogen-free rainbow trout (*Oncorhynchus mykiss*) to detect *Y. ruckeri*, or arctic char (*Salvelinus alpinus*) to detect *A. salmonicida*. Three weeks after addition of rainbow trout, *Y. ruckeri* was cultured from feces and trout started dying from enteric redmouth disease a few days later. Two weeks after addition of char, clinical furunculosis was diagnosed and *A. salmonicida* was isolated from mucus of survivors. After furunculosis or redmouth occurred all trout or char were removed, the entire system was disinfected with 10-ppm chloramines-T, and the system was rinsed with spring water. Specific pathogen-free trout or char were stocked into the system and monitored for redmouth for six weeks. No disease was seen in either salmonid species nor could either pathogen be isolated from fish or biofilters.
- Cipriano, R.C. and G.L. Bullock. 2001. Evaluation of commercially prepared transport systems for non-lethal detection of *Aeromonas salmonicida* in salmonid fish. Page 1 In: 26th Annual Eastern Fish Health Workshop, Clarion Inn Hotel and Conference Center, Shepherdstown, WV.** In vitro studies indicated that commercially prepared transport systems containing Amies, Stuart's, and Cary-Blair media worked equally well in sustaining the viability of the fish pathogen *Aeromonas salmonicida*, which causes furunculosis. The bacterium remained viable without significant increase or decrease in cell numbers for as long as 48 h of incubation at 18-20°C in Stuart's transport medium; consequently, obtaining mucus samples in such tubes were comparable to on-site detection of *A. salmonicida*, by dilution plate counts on Coomassie Brilliant Blue agar. In three different assays of 100 samples of mucus from Atlantic salmon *Salmo salar* infected subclinically with *A. salmonicida*, dilution counts conducted on-site proved more reliable for detecting the pathogen than obtaining the samples in the transport system. In the on-site assays, dilution counts detected the pathogen in 34, 41, and 22 samples, whereas this was

accomplished in only 15, 15, and 3 of the respective samples when the transport system was used. In the additional experiment, Arctic char *Salvelinus alpinus* sustaining a frank epizootic of furunculosis were sampled similarly. Here, too, dilution counts were more predictive of the prevalence of *A. salmonicida* and detected the pathogen in 46 mucus samples; in comparison, only 6 samples collected by using the transport system were positive. We also observed that the transport system supported the growth of the normal mucus bacterial flora. Particularly predominant among these were motile aeromonads and *Pseudomonas fluorescens*. In studies of mixed culture growth, two representatives of both of the latter genera of bacteria outgrew *A. salmonicida*—in some cases, to the total exclusion of the pathogen itself.

- **Hickerson, J., C.E. Starliper and B.C Lidgerding. 1999. Studies on the cohabitation of fresh water bivalves and salmonid fishes: pathology to fish of mussel tissues and contagion of a known fish pathogen, *Aeromonas salmonicida*. 74th Annual Meeting of the West Virginia Academy of Science, April 10, National Conservation Training Center, Shepherdstown, WV.**
- **McAllister, P.E. and J. Bebak. 1997. Infectious pancreatic necrosis virus: detection, distribution and environmental significance in stream water and effluent from salmonid fish hatcheries. Page 93 In: Pathogens and Diseases of Fish in Aquatic Ecosystems: Implications in Fisheries Management. Pacific Northwest Fish Health Protection Committee, June 3-4, Portland, OR.** Effluents from three salmonid fish hatcheries were monitored for the discharge and subsequent downstream distribution of infectious pancreatic necrosis virus (IPNV). Springwater, surface water, hatchery effluent water, and tissues from stream-resident salmonid and nonsalmonid fishes were assayed for IPNV. Virus was not detected in surface water collected above the primary fish hatcheries or in hatchery springwater or reservoir water supplies. IPNV could be detected in fish hatchery effluent (about 1.3×10^4 pfu/l) and from stream water (about 1.0×10^2 pfu/l) for at least 19.3 km (12 mi) below the point of effluent discharge. Effluent from fish hatcheries appeared to be the principal source of IPNV for the streams. The prevalence of IPNV infection in stream-resident salmonid fish was 2.8%, and IPNV was not detected in non-salmonid fish. Adult salmonids and non-salmonids were seemingly not clinically affected by chronic exposure to IPNV, but the effects on stream-resident fry and fingerling salmonids are less clear because of the relative concentration of IPNV recovered from fingerling brook trout (3.0×10^4 pfu/gm). The epizootiological significance of chronic, low-level virus exposure on infection and probability of mortality is under study to determine interactions involving virus concentration, population density, and fish age.
- **McAllister, P.E. and J. Bebak. 1999. Chronic, low-level exposure to infectious pancreatic necrosis virus (IPNV): An estimation of effects on early life stages of rainbow trout, *Oncorhynchus mykiss*. In: 55th Annual Northeast Fish and Wildlife Conference, April 11-14, Manchester, NH.** Previously, we

documented that infectious pancreatic necrosis virus (IPNV) could be detected in stream water for a distance of at least 19.3 kilometers below the point of effluent discharge from a fish hatchery. A low prevalence of IPNV infection was found in stream resident fishes. However, notable IPNV titer (3.0×10^4 plaque forming units (PFU)/gram of tissue), but no clinical signs of disease, was detected in a young trout—ostensibly the progeny of in-stream reproduction. Laboratory trials were conducted to estimate the effects of chronic, low-level IPNV exposure on early life stages of salmonid fish. Using three concentrations of IPNV (10^1 , 10^3 , and 10^5 PFU/liter of water) to simulate conditions found in the affected stream, eyed eggs of rainbow trout (*Oncorhynchus mykiss*) were exposed continuously to IPNV beginning 7 days before hatch, and exposure was continued through alevin, fry, and fingerling life stages until progeny were 44 days old. Cumulative IPNV-related mortality and IPNV-carrier prevalence in survivors were affected by virus concentration and by population density. At low population density (about 35 fish/one liter tank; water flow = 250 milliliters/minute), IPNV-related mortality began as early as 19 days post hatch. As ambient virus concentration increased, average cumulative IPNV-related mortality increasing from 0% to 22%, and average IPNV-carrier prevalence in survivors increasing from 0% to 100%. At high population density (about 140 fish/one liter tank; water flow = 250 milliliters/minute), IPNV infection was detected at 6 days post hatch, and IPNV-related mortality began at 16 days post hatch. As ambient virus concentration increased, average cumulative IPNV-related mortality increased from 1% to 95% and average IPNV-carrier prevalence in survivors increased from 0% to 68%. Ambient virus concentration, population density, and excretion augmentation of ambient virus interact to pressure threshold conditions requisite to initiate and sustain infection and disease in a population.

- **McAllister, Philip E. and Julie Bebak. 1999. Effects of chronic low-level exposure to infectious pancreatic necrosis virus (IPNV) on early life stages of rainbow trout, *Oncorhynchus mykiss*. Page 46 In: 24th Annual Eastern Fish Health Workshop (R. Cipriano, ed.), March 9-11, Atlantic Beach, NC.** In a previous study, we documented that infectious pancreatic necrosis virus (IPNV) was detected in stream water for a distance of at least 19.3 kilometers below the point of effluent discharge from a fish hatchery. A low prevalence of IPNV infection was found in stream resident fishes. However, notable IPNV titer (3.0×10^4 plaque forming units (PFU)/gram of tissue), but no clinical signs of disease, was detected in a young trout—ostensibly the progeny of in-stream reproduction. In the current study, laboratory trials were conducted to estimate the effects of chronic, low-level IPNV exposure on early life stages of rainbow trout, *Oncorhynchus mykiss*. fish. Using three concentrations of IPNV (10^1 , 10^3 , and 10^5 PFU/liter of water) to simulate conditions found in the affected stream, eyed eggs of rainbow trout (*O. mykiss*) were exposed continuously to IPNV beginning 7 days before hatch, and exposure was continued through alevin, fry, and fingerling life stages until progeny were 44 days old. Cumulative IPNV-related mortality and IPNV-carrier prevalence in survivors were affected by virus concentration and by population density. At low population density (about 35

fish/one liter tank; water flow = 250 milliliters/minute), IPNV-related mortality began as early as 19 days post hatch. As ambient virus concentration increased, average cumulative IPNV-related mortality increasing from 0% to 22%, and average IPNV-carrier prevalence in survivors increasing from 0% to 100%. At high population density (about 140 fish/one liter tank; water flow = 250 milliliters/minute), IPNV infection was detected at 6 days post hatch, and IPNV-related mortality began at 16 days post hatch. As ambient virus concentration increased, average cumulative IPNV-related mortality increased from 1% to 95% and average IPNV-carrier prevalence in survivors increased from 0% to 68%. Ambient virus concentration, population density, and excretion augmentation of ambient virus interact to pressure threshold conditions requisite to initiate and sustain infection and disease in a population.

- **Morton, F.M., C.E. Starliper, J. Bebak-Williams. 2001. Furunculosis epidemics in Arctic char: Effects of density and culling. In: 76th Annual West Virginia Academy of Sciences, West Liberty State College, April 21st, West Liberty, WV.** *Aeromonas salmonicida*, a virulent bacterium, is the causal agent of Furunculosis in salmonid fishes. An avoidance and management strategy to reduce the severity of disease includes the daily removal of dead fish that have succumbed to the disease. Moribund fish, those individuals near death, are the most weakened and are carrying the heaviest load of viable bacteria. Moribund fish shed viable bacterial cells, as do dead fish, from mucus and feces into the water column which serves to re-infect the population; however, it is not routine practice to cull moribund fish prior to their death. It is reasonable to speculate that removing moribund individuals will have the benefit to the population that is afforded by picking of mortality. This study was done to evaluate the effects of loading density and culling of moribund fish on the severity of infection and mortality in the population. Arctic char (*Salvelinus alpinus*), a salmonid that is highly susceptible to *A. salmonicida*, were used in all studies. Initial challenges were done with three strains of *A. salmonicida* to determine LD₅₀ values following IP challenges; one, having the lowest LD₅₀ (2.4 cfu/fish) was selected for use in subsequent challenges. Tanks were loaded with pathogen-free char to initial loading densities of 25, 40 or 55 kg/m³ (optimum=52.1 kg/m³). Exceeding the optimum can lead to stressors to fish such as reduced dissolved oxygen, increased nitrogen-ammonia, and increased suspended solids. This density-related stress can leave fish far more susceptible to diseases should pathogens be encountered. Within each density, there was a group of fish with moribund culling and one not culled, which served as the control. All groups of fish were infected by cohabitation with ten fish (per group) that were injected IP with 8.0×10³ cfu per fish. We previously determined that this challenge source (to the pathogen-free fish) would serve to infect, but not cause too high mortality. The results indicate that density and lack of moribund culling can affect the health of a population of fish.
- **Noble, A.C. 1999. Case history: Gill pathologies associated with acute and chronic mortalities in arctic char (*Salvelinus alpinus*) cultured in a semi-**

closed recycle system. Page 5 In: 24th Eastern Fish Health Workshop (R. Cipriano, ed.), March 9-11, Atlantic Beach, NC. A cohort of 2520 arctic char (*Salvelinus alpinus*) with an average size of 270 gms, was introduced into a semi-closed recycle system at a density of 71.6 kg/m³. About two months after introduction, fish stopped gaining weight and feed consumption decreased, followed several days later by no feeding response. Mortalities of a few fish per day occurred for about a week, then increased, peaked at about 225 fish per day and then decreased. Macroscopically, gill filaments had inflamed tips and white patches. Excess mucus and hyperplasia of the gills was observed on wet mounts. All cultures for systemic bacteria, FA tests, and wet mount observations were negative for pathogens. Assays for IPNV, IHNV, VHSV, and ISAV were also negative. Feed samples were negative for rancidity and mycotoxins. Histopathology samples revealed severe chronic-active gill inflammation that was most likely responsible for the high mortalities. All fish in the sample taken during acute mortalities showed a severe chronic diffuse granulocytic and suppurative proliferative branchitis with secondary lamellar fusion and mucus cell hyperplasia. Numerous eosinophilic intranuclear inclusions within primary lamellar epithelial cells were commonly associated with the hyperplasia and inflammation in severely affected fish. These inclusions did not stain as DNA with a Feulgen's stain, and did not appear to resemble viral particles ultrastructurally. In one of the sample groups gill tissue contained irregular granular basophilic intracytoplasmic inclusions stained intensely acidophilic with Gram's stain but did not appear to be bacteria. The same inclusions were red when a Machiavello's stain was utilized, however their morphology and tinctorial properties were not identical to the epitheliocystis inclusions that have been observed in the gills of tilapia. Electron microscopy of the epithelial cells of the gill showed a large, single, centrally-located aggregate of amorphous electron-dense granular material most consistent with nucleolar hypertrophy. Immunohistochemistry performed on gill samples using anti-chlamydial antibodies was negative.

- Spurgeon, A.B., C.E. Starliper and B.C. Lidgerding. 1999. Relative susceptibility of Nauyuk and Labrador Arctic char to experimental challenge with bacterial pathogens: a comparison to rainbow and brook trout. 74th Annual Meeting of the West Virginia Academy of Science, April 10, National Conservation Training Center, Shepherdstown, WV.** Arctic char are becoming commercially important in the United States, primarily in more Southern regions, because of their high conversion rate and high marketability. However, there is very minimal literature as to their susceptibility to the pathogens and diseases routinely encountered in other salmonid hosts. The present study was done to evaluate the virulence of selected bacterial pathogens, including *Aeromonas salmonicida*, to determine relative susceptibilities among four salmonid hosts: Rainbow trout (*Oncorhynchus mykiss*), Brook trout (*Salvelinus fontinalis*), an Nauyuk and Labrador Arctic char (*Salvelinus alpinus*). All sample hosts were fingerlings, similar in size and averaging 8-10g each. Three challenge strains were isolated from naturally occurring epizootics in Brown

(*Salmo trutta*) and Brook trout from the Eastern United States. Isolates of the bacterial pathogen were cultured in tryptic soy broth (TSB) and serial 10-fold dilutions were prepared. For each host, groups of 15 fish were challenged by a 0.1mL intraperitoneal injection (IP) of each dilution. Cultures for challenge were quantified with an average 1.42×10^8 cfu/mL. In the group that received the lowest dilution, each fish was exposed to approximately 1.42×10^6 cfu viable cells. Mortalities within each host were observed over a period of 14-21d. Re-isolation and confirmation of pathogen presence was determined by biochemical evaluation. Brook trout were recognized to be highly susceptible to the pathogen, Nauyuk was second in susceptibility, Labrador third, and Rainbow trout showed highest resistance. The same isolates were introduced to groups of host fish via waterborne, or bath challenges. Each challenge strain was grown in 1L quantities. Groups of 15 fish of each host were challenged with undiluted (10^0) and diluted (10^{-1}) viable culture. Fish were removed from their tanks, placed into challenge culture for 60s, and returned to tanks for observation. Mortalities were recorded, and re-isolation and confirmation was obtained by biochemical testing. These experiments are currently ongoing.

- Hickerson, J., C.E. Starliper and B.C Lidgerding. 1999. Studies on the cohabitation of fresh water bivalves and salmonid fishes: pathology to fish of mussel tissues and contagion of a known fish pathogen, *Aeromonas salmonicida*. 74th Annual Meeting of the West Virginia Academy of Science, April 10, National Conservation Training Center, Shepherdstown, WV.** Currently 71.7% of the known freshwater mussel species native to North America are categorized as endangered, threatened, or of special concern. In 1995, the U.S. Fish and Wildlife Service and other partners initiated conservation efforts to protect native bivalves from zebra mussel infestation and other threats. Native animals are being relocated to refugia, e.g. salmonid fish hatcheries that are void of these threats. These animals are being maintained and efforts at their propagation were established so they can be returned to their native environment at an appropriate future date. With cohabitation of the bivalves and hatchery-reared fish, questions arose regarding the potential for introduction and transmission of pathogens. The present study evaluated the possibility for (nonspecific) mussel tissue toxicity to fish and pathogen contagion between fresh water mussels and salmonids. Tissues were collected from 20 *Amblema plicata*, homogenized and evaluated in vitro using the differential media Blood Agar, Gelatin, and Chondroitin Sulfate. Hemolytic activity was demonstrated in nineteen of the twenty animals and ten were selected for in vivo (intraperitoneal and intramuscular) injections to *Salvelinus alpinus*. No adverse pathology was noted. Contagion between mussels and fish was studied using the bacterium *Aeromonas salmonicida*, the cause of furunculosis in salmonids. *A. plicata* were exposed and infected with the bacterium by either cohabitation with *A. salmonicida* infected fish or by challenge with a viable *A. salmonicida* culture. Infected mussels were then moved to a clean tank and uninfected fish added. Clean fish became infected only when *A. plicata* were exposed to high numbers of

bacteria. Our results indicate minimal chance for contagion from *A. plicata* to salmonids.

- **Starliper, C.E. 2000. Depuration of *Aeromonas salmonicida* by the freshwater bivalve *Amblema plicata* to prevent the bacterium's transmission to Arctic char (*Salvelinus alpinus*). 25th Annual Eastern Fish Health Workshop. April 10-13, Plymouth, Massachusetts.** Freshwater bivalves are very susceptible to environmental and biological factors that impact their existence. Factors such as dams, construction, siltation and most recently the unwanted introduction of zebra mussels (*Dreissena polymorpha*) have resulted in greater than 70% of the animals native to North America to become listed as threatened, endangered or of special concern. In 1995, the U.S. Fish and Wildlife Service, with other partners, initiated conservation efforts; one was a relocation program whereby animals would be collected and propagated at facilities, including fish hatcheries, free of their natural threats with the ultimate goal of reintroduction. With relocation, questions arose regarding the potential to introduce fish pathogens with the mussels. According to standard protocol, prior to relocation mussels must undergo a 30-day quarantine to ensure zebra mussels are not inadvertently spread. The study presented here was done to determine if quarantine in pathogen-free water would also provide for the mussels to depurate fish bacterial pathogens they might harbor. There are no bacterial pathogens known to cause disease to both mussels and fish, therefore, a model system was developed for study utilizing the bivalve *Amblema plicata*, the salmonid fish Arctic char (*Salvelinus alpinus*) and *Aeromonas salmonicida*. An artificial epizootic was established by injecting (i.p.) two groups of Arctic char (60 fish each) with 2.26×10^2 cfu and 2.26×10^0 cfu, respectively, of viable *A. salmonicida* per fish. These fish were placed in a tank with non-injected char, which became infected via cohabitation. The injected fish died and after the non-injected fish began to die, 120 *A. plicata* were placed in the tank. When the *A. plicata* had become carriers of and potential vectors for *A. salmonicida*, the animals were transferred to four tanks and pathogen-free Arctic char were added after 1, 5, 15 and 30 day quarantine periods. Transmission of *A. salmonicida* from the mussels to the "clean" char was then monitored. Infectivity and transmission to fish and mussels was determined by bacteriological culture. It was found that a quarantine period in pathogen-free water is effective to prevent transmission of *A. salmonicida*, but effectiveness depends on the length of quarantine.
- **Starliper, C.E. 2001. A technique for isolation of bacteria from freshwater bivalves. Page 63 In: 2nd Symposium Freshwater Mollusk Conservation Society, Westin Convention Center, March 12-14, Pittsburgh, PA.** One tool in the conservation of freshwater bivalves is a relocation program. Native bivalves are taken from their environment, for example, prior to an advancing infestation with zebra mussels, and then are placed on facilities free of their natural predators. Bivalves are to be maintained and perhaps, propagated, but with the goal of reintroduction at an appropriate future date. Selected safe refuges to maintain bivalves includes hatcheries that intensively rear fishes, particularly

salmonids e.g. trout. With relocation and subsequent co-culture of fish and bivalves, concerns arose regarding the potential for transmission of pathogens. Studies were initiated to address these concerns. Techniques for isolation of bacteria from fish are well established and widely employed, but information on culture of bacteria from freshwater bivalves is sparse. This study describes a technique to reliably isolate aerobic and facultative anaerobic bacteria from bivalves. Fluid (inside valves and outside soft tissues) and soft tissues (portioned as “gut” and “OT”) were collected, weighed, homogenized in peptone-yeast extract, diluted and plated onto primary bacterial isolation media. Soft tissues were surface disinfected with sodium hypochlorite so only the bacteria inside the tissues were isolated. The dilution series employed was effective for isolating bacterial colonies. Bivalves collected from the Ohio River were studied. Total bacterial counts consistently ranged between 1×10^3 and 1×10^6 cfu/g of soft tissue and were dominated by non-fermenters and motile *Aeromonas* spp. Following a relocation, the total counts remained stable, however, the flora profile changed within 24 h.

- **Starliper, C.E., W.B. Schill and J. Mathias. 1998. Evaluation of different serum-free broth media for growth of *Renibacterium salmoninarum*. In: 23rd Annual Eastern Fish Health Workshop, March 31-April 2, Plymouth, MA.** Growth of *Renibacterium salmoninarum* was evaluated in various serum free media, and compared to the serum containing KDM2+M. Fourteen different media were prepared in each trial, and included 12 that were pair-wise comparisons of 6 formulations prepared with and without supplementation with 1% v/v *R. salmoninarum* MCO4M filtered metabolite. Fifty mL cultures were shaken (at 100 rpm) in 250 mL flasks at 15 C and medium performance was evaluated by enumeration of viable cells on days 10, 20 and 30 post inoculation. Three trials were done using starting cell concentrations of 1.13×10^6 cfu/mL per flask (trial 1), 1.23×10^5 cfu/mL (trial 2) and 2.68×10^4 cfu/mL (trial 3). In trials 2 and 3, the pH was estimated using test papers at the same times as cell counts were done. The day that the mean cell yield for each medium was highest (peak day) increased as the inoculum cell concentration was decreased. Eleven of 14 media in trial 1 had a mean peak day on day 10; 9 of the 14 peaked on day 10 in trial 2; and only 2 of 14 in trial 3. For most media, a simple pH determination could be used as a means to check that growth has occurred, particularly useful if charcoal was added directly to media and visual inspection could not be done to detect growth. In general, all media performed well and the choice of which to employ might depend on considerations such as ease of preparation and presence of certain ingredients that might affect subsequent assays.
- **Starliper, C.E., G.L. Bullock, J.A. Hankins and J.A. Mathias, 1998. Relative susceptibility of Arctic char *Salvelinus alpinus* to experimental challenge with selected bacterial fish pathogens. In: 23rd Annual Eastern Fish Health Workshop, March 31- April 2, Plymouth, MA.** Arctic char *Salvelinus alpinus* is a salmonid fish common to colder regions of northern North America. Increasingly, they are being intensively cultured for sport fisheries and as a

marketable food fish, particularly in more southern regions where salmon and trout species are the more prevalent cultured salmonids. Along with this, is increased potential for disease and epizootics, particularly in warmer water temperatures, which are more advantageous to some bacterial pathogens. This study was initiated to evaluate the susceptibility of Arctic char, relative to rainbow *Oncorhynchus mykiss* and brook *Salvelinus fontinalis* trout, to experimental challenge with the bacterial pathogens: *Aeromonas salmonicida*, cause of furunculosis; *Renibacterium salmoninarum*, cause of bacterial kidney disease; and biotypes I and II *Yersinia ruckeri*, cause of enteric redmouth. Groups of fish were exposed by bath and intraperitoneal injection (*R. salmoninarum*, injection only). Water temperature for all challenges was 12.5 C. Observation periods (14-70 d) followed each challenge; mortalities were recorded and death to each pathogen confirmed by biochemical or serological methods. Total mortality of Arctic char due to *R. salmoninarum* and *A. salmonicida* was similar to brook trout, and both hosts were more susceptible than rainbow trout. Mortality to both biotypes of *Y. ruckeri* was similarly high for Arctic char and rainbow trout, and contrasted to relatively low mortality in brook trout.

- **Yeakle, B. and C.E. Starliper. 2000. A susceptibility comparison of two strains of Arctic char and rainbow and brook trout to experimental challenge with the fish pathogen *Yersinia ruckeri*. 75th Annual Meeting of the West Virginia Academy of Science. April 15. Fairmont State College, Fairmont, West Virginia.** *Yersinia ruckeri* is a Gram-negative, enteric bacterium that causes enteric red mouth disease (ERM), a very serious disease that affects salmonid fishes and may result in high mortality. The two salmonid species that are the most frequently intensively cultured in the United States are rainbow(*Oncorhynchus mykiss*) and brook (*Salvelinus fontinalis*) trout. Previous disease case histories and experimental challenge data have shown that rainbow trout are relatively more susceptible to *Y. ruckeri* than brook trout, about a 1,000-fold difference. The study we present was done to experimentally evaluate and compare the susceptibility to *Y. ruckeri* of the Nauyuk and Labrador strains of Arctic char (*S. alpinus*), to each other, and to rainbow and brook trout. Arctic char are naturally a northern, cold-climate species. In recent years culturists have increasingly begun to intensively rear Arctic char in the warmer waters of more southern geographic areas as the species is becoming more commercially important in aquaculture because of their very favorable conversion rate to convert fish feed into edible filets. Little is known of the susceptibility of Arctic char to the major, primary bacterial pathogens of salmonids, however, with increased intensive culture diseases may be predicted to emerge caused by recognized salmonid pathogens or perhaps, to yet to be described pathogens. We artificially challenged groups of fingerlings of each of the four hosts by an intraperitoneal (IP) injection and by waterborne (bath) exposure. Then, the fish were observed, mortality was recorded and dead fish were necropsied to show death was a result of *Y. ruckeri* infection. The bacterium was isolated by streak culture of kidney tissues onto brain heart infusion (BHI) agar; bacterial isolates were characterized using standard biochemical methods and media. For the IP

challenges, each *Y. ruckeri* was grown in BHI then ten-fold dilutions were prepared and 0.1 mL of each dilution was injected per fish. For the waterborne challenge, isolates were grown in BHI and fish were exposed for 1 min to full strength and 10^{-1} concentrations of viable cells. All groups (of 15 fish each) were maintained in flow through tanks fed with 12°C pathogen-free spring water at about 1.5 L per minute. Six *Y. ruckeri* isolates were utilized; three were of the biotype I (sorbitol fermentation negative) and three were biotype II (sorbitol positive). The two biotypes were examined because some earlier reports in the literature noted that biotype II strains were significantly less virulent than biotype I strains. Both strains of Arctic char were very susceptible to both biotypes of *Y. ruckeri* by both challenge methods of exposure. With some challenges the Nauyuk strain was minimally more susceptible at the higher dilutions than the Labradors in terms of mortality and time to mortality. The susceptibility of the rainbow trout was similar to that for the two Arctic char hosts. Of the four hosts, brook trout were relatively far more resistant to *Y. ruckeri* challenge, regardless of method.

- **Starliper, C.E. 2001. Transmission, via *Amblema plicata*, of the fish pathogen *Aeromonas salmonicida* to Arctic char is interrupted by quarantine of the bivalves. Second Symposium of the Freshwater Mollusk Conservation Society. Pittsburgh, PA. March 12-14.** A relocation program is one strategy of the conservation efforts employed to protect native freshwater bivalves. With relocation, native bivalves are removed from their natural environment prior to the detrimental effects of advancing zebra mussels. Then, they are placed in threat-free facilities for maintenance and propagation with the ultimate goal of reintroduction at appropriate future date. Holding facilities include hatcheries that intensively rear salmonid fishes and this led to concerns regarding the potential to introduce fish pathogens via the relocated native bivalves. After collection and prior to relocation bivalves undergo a minimum 30-day quarantine to ensure zebra mussels are not inadvertently spread. This study was done to determine if a quarantine would also provide for bivalves to depurate fish bacterial pathogens they might be harboring. A model system was developed for study utilizing the bivalve *Amblema plicata*, the salmonid fish Arctic char (*Salvelinus alpinus*) and *Aeromonas salmonicida*, the bacterium that causes furunculosis disease in salmonids. Clinical furunculosis was established by injecting (IP) two groups of Arctic char (60 fish each) with 2.26×10^2 or 2.26×10^0 of viable *A. salmonicida* cells per fish. These fish were placed in a tank with non-injected char, which became infected via cohabitation. Once the non-injected fish began to die, 120 *A. plicata* were placed in the tank. When the *A. plicata* had become carriers of, and potential vectors for *A. salmonicida*, they were transferred to four different tanks and pathogen-free Arctic char were added after the *A. plicata* were allowed to depurate for 1, 5, 15 or 30 days. Transmission of *A. salmonicida* from *A. plicata* to char was determined by bacterial culture. It was found that quarantine period of sometime less than 15 days was effective in preventing transmission of the bacterial fish pathogen.

- McKee, E. and C.E. Starliper. 2000. The effect of quarantine of freshwater bivalves (e.g. *Amblema plicata*) to control transmission of the fish pathogen *Aeromonas salmonicida* to salmonid fishes. 75th Annual Meeting of the West Virginia Academy of Science. April 15. Fairmont State College, Fairmont, West Virginia.** Freshwater bivalves are very susceptible to environmental and biological factors that impact their existence. Factors such as dams, construction, siltation and most recently the unwanted introduction of zebra mussels (*Dreissena polymorpha*) have resulted in greater than 70% of the animals native to North America to become listed as threatened, endangered or of special concern. Bivalves require, as part of their life cycle, an intermediate fish host for development of glochidia into the juvenile stage. Therefore, impacts on the fish directly affect survival of mussels. In 1995, the U.S. Fish and Wildlife Service with private, state and other federal partners initiated efforts to conserve native mussels. One of these conservation efforts was a relocation program whereby animals would be collected and propagated at facilities free of their natural threats. Facilities include those that already rear salmonid fishes. With relocation, questions arose regarding the potential to introduce fish pathogens with the mussels. According to standard protocol, prior to relocation mussels must undergo a 30-day quarantine to ensure zebra mussels are not present and inadvertently spread. The study presented here was done to determine if quarantine in pathogen-free water would also provide for the mussels to depurate fish bacterial pathogens they might be harboring from being in their natural environment. There are no bacterial pathogens known to cause disease to both mussels and fish, therefore, a model system was developed using the bivalve *Amblema plicata*, the salmonid fish Arctic char (*Salvelinus alpinus*) and *Aeromonas salmonicida*, a Gram-negative bacterium that is pathogenic to salmonids. An artificial epizootic was established by injecting (IP) two groups of Arctic char with 2.26×10^2 cfu and 2.26×10^0 cfu viable *A. salmonicida* per fish. These fish were placed in a tank with non-injected char, which became infected via cohabitation. The injected fish died and after the non-injected fish began to die, 120 *A. plicata* were placed in the tank. When the *A. plicata* had become carriers of, and potential vectors for *A. salmonicida*, as determined by bacteriological culture, the animals were transferred to four different tanks and pathogen-free Arctic char were added after 1, 5, 15 and 30 day quarantine periods. Transmission of *A. salmonicida* from the mussels to the “clean” char was evaluated through subsequent disease and mortality followed by bacteriological isolation and characterization of *A. salmonicida* from mucus and kidney tissues. In those groups where disease did not develop bacterial culture of *A. salmonicida* from fish and mussel tissues was done to determine if the bacterium was present and its prevalence in both hosts. A quarantine period in pathogen-free water is effective to prevent transmission of *A. salmonicida*, but effectiveness is dependant on the length of the quarantine.
- Starliper, C.E., J. Mathias and J. Hickerson. 1999. Interaction of freshwater unionids and salmonid fishes: relevance of bacterial contagion. Freshwater Mollusk Conservation Society, First Annual Symposium “Musseling in on Biodiversity”. Chattanooga, Tennessee. March 17-19.** An aspect of native

unionid conservation is to relocated them to refugia, which have included salmonid fish hatcheries. With this, questions arose regarding the potential for introduction and contagion of pathogen between unionids and fish. The information presented will summarize methodology and results of several studies we have conducted in an effort to address these questions. A simple method for isolation and quantification of bacteria will be described. Total bacteria of unionid soft tissue were quite stable (about 1×10^5 cfu/g), however, the distribution of bacterial types changes relative to water supply. Unionid soft tissue(s) demonstrated in-vitro hemolysin-like activity, but no apparent pathology when used in IP and IM injection challenges of fish. Extensive efforts were made to isolate fish pathogenic bacteria from non-quarantined, native bivalves during the 1997 sampling season. Only environmental-opportunistic pathogens were recovered, however, in a previous study, *Flavobacterium columnare*, cause of columnaris disease in many fish species, was isolated from a single *Amblema plicata*. Bacterial challenges involving *A. plicata* and salmonid fishes will be discussed; specifically, exposure of *A. plicata* to “normal” bacterial flora of fish, and laboratory model for pathogen transfer using the fish pathogen *Aeromonas salmonicida* with which pathogen contagion was demonstrated in laboratory studies involving *A. plicata* and arctic char, *Salvelinus alpinus*.

- **Starliper, CE, R. Vilella, P. Morrison and J. Mathias. 1998. Preliminary studies on the potential for bacterial pathogen contagion between freshwater mussels and salmonid fish. Freshwater Mussel Symposium. March 6-8. Columbus, Ohio.** Over 70% of approximately 300 species and subspecies of freshwater mussels native to the United States are categorized as endangered, threatened or of special concern. There are a number of factors contributing to the decline and include those that affect mussels and their fish hosts. In recent years a new threat to native populations in many large river systems has been the introduction of the zebra mussel, *Dreissena polymorpha*. Because of the combined threats to native populations, effort was initiated in 1995 by the U.S. Fish and Wildlife Service and other federal, state and private partners to collect and hold native animals from large rivers in areas of zebra mussel infestation. These individuals would be maintained and propagated in some federal facilities, including fish hatcheries that rear salmonid fishes such as rainbow, *Oncorhynchus mykiss* and brook trout, *Salvelinus fontinalis*. With this relocation, questions arose regarding the potential for transmission of pathogenic microorganisms from mussels to fish, or fish to mussels. Our studies were initiated to evaluate and study the bacterial flora of common, native freshwater mussels collected from the Ohio River. Techniques of bacterial sampling were presented and tissues were used to inoculate a battery of general and specific media for isolation of salmonid fish pathogenic bacteria.
- **Starliper, C.E., R. Vilella, P. Morrison and J. Mathias. 1997. Sampling the bacterial flora of freshwater mussels from the Ohio River. 22nd Annual Eastern Fish Health Workshop. Atlantic Beach, NC. Mar 18-20.** There are 300 different species of freshwater mussels native to North America and over

70% are categorized as endangered, threatened or of special concern. This and zebra mussel (*Dreissena polymorpha*) infestations, caused the USFWS and other partners to collect and propagate native mussels in certain federal hatcheries with a goal of reintroduction into their native habitat at an appropriate future date. Thus, questions arose regarding contagion of bacterial pathogens between mussels and fish. The information presented here involves ongoing studies to answer such questions. Mussels native to the Ohio River (mile 175, Wood County, WV) were collected by brailing in November 1995, and June and September, 1996. In initial studies, we wanted to determine if (1) any salmonid pathogenic bacteria could be isolated from the mussels and (2) to evaluate the stability of their bacterial flora. Soft tissues were collected and separated into two samples, gut and all other tissues combined as one. Tissue samples were diluted and homogenized, then used to inoculate primary isolation media. Resulting bacteria were enumerated and representative colonies subculture for identification. The distribution of bacteria was somewhat equal between gut and all other tissues combined. Total bacterial counts remained relatively stable over time. Of 1153 bacteria isolated, motile *Aeromonas* spp. (26%) and the nonfermenter group (46%) were predominant. *Flavobacterium columnare* was isolated from one mussel in June 1996.

D. Technology transfer

1. Industry workshops and meetings

- Bebak, J. 1997. Fish health management: the whole farm approach. National Association for State Aquaculture Coordinators, May 29th, Harrisburg, PA. (No abstract)
- Bebak, J. 1998. Biosecurity/fish health management practices. In: Management Principles for Coldwater Fish Production, Freshwater Institute and Shepherd College, July 30-August 1, Shepherdstown, WV. (No abstract)
- Bebak, J. 1998. Protozoal diseases of freshwater finfish. In: Management Principles for Coldwater Fish Production, Freshwater Institute and Shepherd College, July 30-August 1, Shepherdstown, WV. (No abstract)
- Bebak, J. 2000. Biosecurity: What is it? How do you get started? 12th Annual Minnesota Aquaculture Conference and Trade Show. Minnesota Aquaculture Association, February 18-19, Hinckley, MN. (No abstract)
- Bebak, J. 2000. Arctic char: What is the potential? 12th Annual Minnesota Aquaculture Conference and Trade Show. Minnesota Aquaculture Association, February 18-19, Hinckley, MN. (No abstract)
- Bebak-Williams, J., 2001. Veterinary Epidemiological Perspective in Aquaculture. Wisconsin Aquaculture Conference, Marriott Madison West Hotel and Convention Center, March 21-24, Madison, WI. (No abstract)

Bullock, G.L. 1998. Bacterial diseases of freshwater finfish. In: Management Principles for Coldwater Fish Production, Freshwater Institute and Shepherd College, July 30-August 1, Shepherdstown, WV. (No abstract)

Hankins, J.A. 1998. Aquaculture permitting issues. In: Management Principles for Coldwater Fish Production, Freshwater Institute and Shepherd College, July 30-August 1, Shepherdstown, WV. (No abstract)

Noble, A.C. 1998. Options for treatment. In: Management Principles for Coldwater Fish Production, Freshwater Institute and Shepherd College, July 30-August 1, Shepherdstown, WV. (No abstract)

2. West Virginia Aqua

Soon after the inception of this project, FI staff began transferring technology/information about Arctic char rearing systems, culture requirements and management strategies to the Mingo County Redevelopment Authority (MCRA), an economic development agency. MCRA contracted with the FI to find a site with suitable conditions for an aquaculture facility. FI staff designed the hatchery, which is capable of producing 500,000 to 1,000,000 fingerlings per year. FI staff made regular site visits, oversaw key aspects of fish production system construction, compiled a comprehensive operating manual for the char hatchery and provided hands-on training to employees. Facilities for char grow-out have been constructed and FI staff continue to provide technical assistance regarding design and/or operation of the hatchery and grow-out facilities.

3. Quarterly reports

Four times per year, quarterly reports were mailed to active and potential char producers in the U.S. and Canada. These reports were also sent to aquaculture extension agents and research scientists.

4. Telephone

Technology transfer via telephone calls is a significant part of technology transfer at the Freshwater Institute. FI researchers answer questions about char culture systems and management on an at least weekly basis from producers, suppliers to the industry, extension agents and other researchers.

E. Theses and dissertations resulting from this project

- Jittinandana, S. 2000. Ante- and Post-Mortem Factors Affect Muscle Protein Functionality from Fish. Ph.D. Dissertation, West Virginia University, Morgantown.

F. Collaborators

- Ana Baya, PhD, Head, Bacteriology Section, Maryland Department of Agriculture Animal Health Laboratory, College Park, MD
- Dr. Raymond Boston, Professor, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA
- Mary Carson, PhD, Chemist, U.S. Food and Drug Administration, Laurel, MD
- Rocco Cipriano, PhD, Microbiologist, National Fish Health Research Laboratory, Leetown, WV
- Peter Cooke, PhD, Biologist, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA
- Sitima Jittinandana, Graduate Student, West Virginia University, Morgantown, WV
- P. Brett Kenney, Ph.D., Associate Professor, West Virginia University, Morgantown, WV
- Burt Lidgerding, PhD., Professor, Shepherd College, Shepherdstown, WV
- Patricia Mazik, Ph.D., Unit Leader, WV Cooperative Fish and Wildlife Research Unit, West Virginia University, Morgantown
- Philip McAllister, PhD, Microbiologist, USGS National Fish Health Research Laboratory, Leetown, WV
- Erin McKee, Undergraduate, Shepherd College, Shepherdstown, WV
- Caird Rexroad, PhD, Research Molecular Geneticist, National Center for Cool- and Cold-water Aquaculture, Leetown, WV
- Bane Schill, Research Chemist, USGS National Fish Health Research Laboratory, Leetown, WV
- Dr. Gary Smith, Professor, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA
- Amy Spurgeon, Undergraduate, Shepherd College, Shepherdstown, WV
- Cliff Starliper, PhD, Microbiologist, USGS National Fish Health Research Laboratory, Leetown, WV
- Bruce Wagner, Statistician, U.S. Department of Agriculture Animal Plant Health and Inspection Service, Fort Collins, CO
- Bradley Yeakle, Undergraduate, Shepherd College, Shepherdstown, WV