Aquaculture Food and Marketing Development Project.
Progress Report, January 2002

FY 1998 (Grant 1).

Objective 1. Develop marketing strategies for aquaculture (primarily trout) producers and processors.

Tasks 1-1 and 1-2.

1. Three fee-fishing surveys have been completed.

    a. The first survey was conducted on-site at a various fee-fishing locations and was done utilizing personal interviews of fisherman. The responses from these surveys were used to further develop the mail survey sent to the sample of fishermen holding a West Virginia fishing license.
    b. The second survey obtained information regarding the fishing habits of West Virginia residents who have a West Virginia fishing license. This survey also addressed the respondent's attitudes toward fee fishing as a potential alternative for recreational fishing.
    c. The third survey obtained information regarding fishing habits of nonresidents who have a West Virginia fishing license. This survey also addressed the respondent's attitudes toward fee fishing as a potential alternative for recreational fishing.

2. The data from these two surveys has been compiled and is being analyzed with a draft written report submitted for review and comment.

3. One paper has been submitted for publication consideration at a refereed outlet.

Tasks 1-3 and 1-4

1. A survey for processed fish is underway. A questionnaire has been designed which will be administered to distributors of processed fresh fish products. Currently a list of distributors is being identified. Obtaining this list has been difficult. The purpose of this survey is to assess the demand and the nature of that demand for processed fresh water fish, particularly trout. Once the data has been collected it will be analyzed and a report written.

Objective 2. Examine the economic and financial feasibility of alternative aquaculture species focusing on hill land.

Detailed farm-level trout enterprise budgets were developed using a combination of data from producer mail surveys and published secondary sources. Two types of systems were analyzed, fiberglass tanks and raceways. Given our assumptions, costs per pound were lowest for raceways compared to tanks across all sizes investigated (from 2,500 lbs. to 100,000 lbs. per year). In addition, economies of size were found to exist for raceways, but not necessarily for tanks. In general, results show that trout production in WV and similar hill-land areas can be profitable given current market conditions. Feed, typically represents the largest component of total costs; therefore, profitability is very sensitive to changes in feed costs. Feed management not only has profound implications for

profitability but also for waste management, something that continues to be explored in subsequent phases of the project.

In addition to analyzing and documenting the costs and returns of trout production, the economics of trout processing in hill country was analyzed and documented. A combination of surveys, site visits, and published sources was used. Two plant sizes were investigated, small and large. Results identify break-even processing quantities and illustrate the conditions under which processing can be profitable. Results show that, other than the cost of purchasing the fish itself, labor is the largest component of processing costs. In addition, it was found that, given their current product mix, break-even quantities were relatively large. One strategy to reduce break-even quantities – and increase profits – is to increase the proportion of high-profit products (such as boned-head removed in the case of the small plant) and/or value-added products (such as smoked fillet or pate in the case of the large plant) in the product mix. Alternatively, on the cost side, reducing labor costs -- or increasing labor productivity – represents a strategy for enhancing processing profitability.

Objective 3. To determine the effect of water quality and stress on the consistency and quality of fresh trout fillets and value-added smoked trout products.

Stress and Fillet Quality. The project goal is to increase production of consistent, high quality rainbow trout in West Virginia. Elevated, dissolved carbon dioxide levels are a primary concern and water quality limitation among current aquaculturists throughout the state. Excessive carbon dioxide can interfere with metabolic (suppressed growth), osmotic (pH and ion imbalance), and respiratory (gas exchange) functions of fish. Studies were developed from common farm conditions and practices to simulate the grow-out, handling, and harvesting methods of farm-raised rainbow trout. Three levels of CO_2 (<25 mg/L, 35±5 mg/L, and 45 mg/L) were applied to 5 tanks of fish. These fish were sampled initially, and at 28, 56, and 84 days. Physiological stress responses (blood hematocrit and plasma glucose, cortisol, and chloride), whole fish and fillet weights, initial and ultimate pH, smokehouse yields, fillet shear, expressible moisture, and water-phase salt, and proximate composition were determined.

Total mean growth (average weight gain over 3 months) of fish exposed to high carbon dioxide levels (45±5 mg/L) were significantly less (p-value < 0.001) than fish exposed to either intermediate (35±5) or low (<25) levels. Thus, at the conclusion of the study, fish grown in higher carbon dioxide levels weighed significantly less. Chloride concentrations of fish were significantly (p-value < 0.01) lower in the high CO2 treatment groups compared to fish in the intermediate or low treatment groups. This indicates a decreased ability of fish to maintain optimal blood chloride concentrations at higher CO2 levels. As CO_2 increased, fillet weights decreased and shear force increased. Ultimate pH was not affected by CO_2 level; however, it decreased with increased time. Water retention by the fillets, water-phase salt, cooked moisture, and fat content were not affected by CO_2 level. Smokehouse yield and fat content increased and shear force decreased with increased time on the study. These latter effects are likely associated with an increase in the size of the fillet.

Cryoprotection of Trout Fillets. Deterioration of food quality results from frozen storage, and fish muscle is particularly susceptible to this deterioration. Little information exists for cryopreservation of intact fillets; whereas, considerable work has been done with the minced fish product, Surimi. Sodium lactate and sucrose/sorbitol, alone or with food-grade phosphates or $MgCl_2$, were evaluated for there

ability to preserve the quality of fillets during frozen storage for 90 days at -20 °C. Fillets were soaked in specified cryoprotectant solutions for 90 min. Water was used as a control for the cryoprotectant soaks. Smoked fillets and trout mince were prepared, and muscle color, raw and cooked pH, brine uptake, cook yield, shear force, salt content, water-phase salt content, and proximate composition were measured. Gel hardness and cohesiveness were determined on the fish mince.

Lightness and redness of intact fillets prior to brining and smoking of treated fillets were lower than untreated fillets prior to freezing ($P<0.05$). Sucrose/sorbitol and sodium lactate increased ($P<0.05$) gel hardness and cohesiveness, cook yield, pH and fat content of smoked products compared to an opposite effect for water after frozen storage. A greater increase in cook yield and cooked moisture content was effected by sucrose/sorbitol than by sodium lactate ($P<0.05$). Phosphates increased ($P<0.05$) pH of fillets after soaking that in turn decreased lightness ($P<0.10$) and increased yellowness of the fillets as well as cooked pH ($P<0.10$). Magnesium chloride enhanced ($P<0.05$) the increase in cooked pH caused by frozen storage. Frozen storage increased ($P<0.05$) salt content, water-phase salt content, raw and cooked ash, and decreased ($P<0.05$) brine uptake and fillet shear force. Cryoprotectant minimized the negative effects of frozen storage on intact trout fillets.

Survey of fish farms in WV and VA. Fish processors rely on fish they receive from aquaculture farms. High quality, consistent fish are important to ensuring a marketable product. Many of the variables affecting these fish are on farm variables. Water quality, water source(s), management practices (sanitation, grading techniques, method of feeding, feeding times, harvesting techniques), feed type, genetics of fish selected for production, production system, and stress all impact fish quality. Controlling these variables becomes increasingly important when a coop system is utilized. The coop will be receiving fish from a variety of sources and, it needs fish that are of consistent quality.

In order to determine to what extent these variables impact quality and quality variation, we visited area aquaculture producers and solicited their help. Farm visits were conducted, and a questionnaire was completed that detailed aspects of the farm. Follow up visits entailed water and fish sampling. Due to logistics and limitations regarding freshness of fish samples, only a few farms were sampled per trip. To date, two sampling trips have been undertaken. Trip one on July 19, 1999 included four aquaculture sites in WV and one in VA. Trip two, Oct. 5, 1999, involved two sites in VA and one in WV. Follow-up trips to previously visited sites and new sites are planned in the future. Water and fish samples were transferred to University facilities for analysis. Water was analyzed at the National Research Center for Coal and Energy (NRCCE). Attributes that were analyzed included alkalinity, acidity, Ca, Mg, Mn, Al, NO_2, NO_3, Free CO_2, NH_3, TDS, TSS, Hardness, Zn, and Cu. When water analysis is complete, *we* share the results with the *producer*. In return for their cooperation, the producer receives a free water test. Producers may also receive any and all other information gathered relative to *their* fish. Ten fish were purchased from each farm for analysis. We attempted to purchase fish as close to the same size and age as possible. Once fish were harvested, they were bagged, identified, and placed on ice. Upon returning to the university, each fish was tagged, with respect to its origin, and assigned an ID number. Each fish was then weighed, filleted, and weighed again to determine dressing percent. The pH was also taken at the anterior and posterior portions of each fish. Two fish per farm were randomly selected for raw product analyses. Fresh fish attributes measured were proximate analysis (moisture, fat, protein, and ash), color, and expressible moisture. Sample preparation entailed freezing each sample in liquid nitrogen and powdering it. Samples were also sent to NRCCE for tissue mineral determination.

The remaining eight fish were brined and smoked. Fish were placed in a 33° brine (with addition of brown sugar at 69% of NaCl) for 90 minutes. Subsequently, they were placed on smoke racks and allowed to dry for 24 hours. Fish were left uncovered during the first two hours to allow pellicle formation. The pellicle is a smooth, dry, glossy coating on the outside of the fish resulting from dissolved proteins. A properly formed pellicle allows smoke to adhere better to the surface of the product, and it holds in product juices during cooking. After drying, fish were smoked. Smoked product attributes evaluated were color, brine uptake, cook yield, expressible moisture, proximate analysis, texture, and water-phase salt (wps). Sample preparation involved processing one side chosen randomly each fish. This side was finely chopped in a Quisinart, food processor. The remaining side was used for texture and wps.

Our tests demonstrate, as was hypothesized, that large variations exist for attributes measured. Table one reports raw product characteristics, and table two; differences in cooked product characteristics. Table three lists analyzed water traits. It is challenging to determine the causes of these variations. After all, a trout is a trout; they should all be basically the same. Our producer surveys revealed that the same basic genetic strains of trout are being grown (Kamloops). The surveys also show that farmers are utilizing the same feed source (Ziegler). Because nutrition and genetics are similar, differences may be associated with other variables.

Farmers have a choice of feeding method and amount of feed fed. The amount of feed that fish consume will effect the composition of the fish, which in turn effects dressing percent and further processed fish characteristics. The type of production system may effect composition. An example would be water flow rate. A fish exposed to faster moving water will expend more energy, reduce fat stored, and have a firmer texture. Chronic stress incurred via management practices, water quality, or other means may affect the trout. Chronic stress can cause the fish to eat less and make it more susceptible to disease. A fish's attempt to maintain mineral homeostasis relative to the water it survives in, is an example of this stress. Stress incurred at harvest affects the trout. Stress at harvesting can lead to changes in postmortem metabolism and thus carcass quality and processing characteristics.

To date, our results show that raising the same fish and feeding the same feed does not guarantee consistency. Other factors play a critical role in the conversion of muscle to meat and the resulting meat characteristics.

Objective 4.- Conduct a technology transfer component to disseminate information generated by this project to the aquaculture industry in Appalachia, to state agencies with aquaculture related responsibilities and to the general public.

The following activities were completed:

Updated the listing of West Virginia producers and placed it on the WVU Aquaculture web page (http://www.wvu.edu/~agexten/aquaculture/aquacult.htm) and developed web page as a reference source;

Initiated an exchange with the trout industry in North Carolina with the intent to determine how trout growers in West Virginia can produce trout as a food fish for $1/lb or less. This exchange is aimed primarily at development of mine water sources in the southern part of the state.

Hosted of a state wide meeting of aquaculture interests on January 8, 2000 in Flatwoods, West Virginia featuring investigators from each objective described aquaculture research at WVU. Producers from Illinois, North Carolina, and Colorado shared information regarding problems and opportunities facing their aquaculture businesses. Approximately 70 people attended the one day event. Overall evaluation was 4.34 on a 5.0 scale where 5 = Excellent.

Conducted "road trips" with producers and/or supporters of the West Virginia aquaculture industry to North Carolina, Kentucky, Ohio, Virginia, and Pennsylvania.
Responded to approximately 140 requests for information were fielded from specialists, agents, and individuals in the state and region.

Developed a Brochure describing the Aquaculture Food and Marketing Development Project and distributed it to stakeholders via mailing, at meetings and during personal visits.

The Food Science laboratory provides resource personnel and analytical support to the two the two largest processors of farm raised fish in West Virginia.

Output from work conducted in this grant include:

Costs and Returns of Trout Processing by High Appalachian: A Case Study, by San et al., Summer 2000.

Fidler, Frank 2000. "MA & PA Cooperative: A Case Study," (a draft version was presented to the Board of Directors of Mountain Aquaculture and Producers Association at their Fall annual meeting in Elkins, WV, in December 1999). A follow-up to this case study was conducted by Sztroin and Fincham in Summer 2000.

"Aquaculture 2000," Poster prepared by Dan Miller, for displays at venues such as the WV Extension Service Aquaculture Forum held in Flatwoods, WV, January 8, 2000.

San, Nu Nu, Dan Miller, Gerard D'Souza, Dennis K. Smith, and Ken Semmens, 2001. West Virginia Trout Enterprise Budgets. Version 2.0. West Virginia University Extension Service. Pub. # AQ01-1.

Jittinandana, S., P. B. Kenney, and R. Kiser. 2000. Chemical and Physical Changes in Smoked Rainbow Trout Associated with Frozen Storage. 53rd Reciprocal Meats Conference. Columbus, OH.

Danley, M., P. Mazik, P. B. Kenney, R. Kiser, and J. Hankins. 2001. Chronic exposure to carbon dioxide: growth, physiological stress response, and fillet quality of rainbow trout. World Aquaculture Society Annual Meeting. Orlando, FL.

Jittinandana, S., P. B. Kenney, S. Slider, and R. Kiser. 2001. Cryoprotection of rainbow trout, *Oncorhynchus mykiss*, fillets for smoked trout production. Institute of Food Technologists Annual Meeting and Food Expo. New Orleans, LA.

Jittinandana, S., P. B. Kenney, S. Slider, and R. Kiser. 2001. Effects of cryoprotectants on physicochemical attributes of intact rainbow trout, *Oncorhynchus mykiss*, fillets. Institute of Food Technologists Annual Meeting and Food Expo. New Orleans, LA.

FY 1999 (Grant 2).

Objective 1. - Implement the marketing strategies developed in the FY1998 project for aquaculture producers and processors and assess further opportunities for developing a sustainable aquaculture industry in a multi-state area including the Appalachian region.

Task 1-1: Implement a Market Driven Network for Appalachian Aquaculture

1. Based on the data received from Special Grant Phase 1, Task 1-1 a questionnaire was designed to collect data from both West Virginia residents with a West Virginia fishing license and non-residents with a West Virginia fishing license. Those interviewed in this survey included all those respondents from Grant #1, Task #1 who expressed an interest in fee fishing in West Virginia and in participating in a fee fishing package in West Virginia. The questionnaire for the survey has been completed, tested, and mailed to the respondents. The data is currently being compiled with a written report to follow.

2. Personal interviews of 90% of the fee-fishing operators in West Virginia has been conducted in order to assess what is currently available to fishermen wanting to participate in a fee-fishing experience. The data from these interviews will be compared to the data gathered from the licensed fishermen mentioned above.

Task 1-2 Implement a Cooperative Approach to Marketing

The researchers have met with organizations which can play a role in developing a cooperative marketing approach to processing West Virginia's aquaculture products. The opportunities surrounding this approach are currently being pursued with these organizations.

Task 1-3: Recreational Fee Fishing Utilizing Streams

Both residential and non-residential West Virginia fishing license holders have been surveyed to determine the level of interest and the market potential for recreational fee fishing utilizing streams. The data has been collected and is now being analyzed with a report to follow.

Objective 2. – Economic Analysis. To determine the expected costs and returns of producing and processing aquaculture products suitable to hill country such as that in west Virginia ant to examine other issues relating to the development of a sustainable aquaculture industry in the study area including identification of suitable water supply sources, waste management practices, and economic development impacts of aquaculture.

2.1 Farm level and processor level optimization models.

Estimation of two separate models, (a) farm-level and (b) processor-level economic efficiency models using data from the surveys (conducted as part of grant 1) and from published sources has been completed. In addition, case studies of each of the two major aquaculture processors in the study area were completed. The results have been documented, and publications listed in the last progress report. In terms of stakeholder impact, available evidence to date suggests that, in the case of both processors, results of the case studies and resulting economic analyses were used to improve operating and sales procedures, which, in turn, led to improved financial performance.

2.2 – Assess Economic Impacts and Waste Management Options.

To analyze statewide economic impacts from expansion in the aquaculture industry, an economic multiplier analysis was initiated during this grant period. The software package IMPLAN, which is useful for analyzing economic impacts at the county or statewide level, was used for this purpose. In addition, an analysis of waste management options was undertaken. This analysis shows that factors such as the type of feed (pelleted vs. extruded), and how and when the feed is administered are important determinants of the amount of waste, and, therefore, the cost of waste disposal. Previous studies of the study area show that filtration was the most cost-effective waste disposal option, adding approximately $0.05 per pound to trout production cost; alternatively, if untreated, downstream costs (or external costs) could be substantially greater, amounting to $0.22 per pound of trout. This has implications for both producers and policy makers. Further analysis of waste disposal options for the study area, and costs thereof, depend on the availability of data from the on-going waste management studies by other participants in this project.

2.3 – Assess Mine water sources suitable for economical production of food size trout in West Virginia.

The GIS analysis of mine-water sources suitable for aquaculture in WV was completed and is updated periodically as new information becomes available from site visits, water quality tests, and on-going bio-assays from the cooperative agreements with local coal mining companies at various sites around the study area. Ideally, three or more years of production data will be needed to conduct an economic analysis. Since the bioassays are nearing the end of the first year, it is not possible to obtain results from the economic analysis.

Objective 3. Product Quality Research. Improve the consistency and quality of fresh trout fillets through improved feeding and harvesting techniques.

Effect of water velocity and feeding rate on growth and fillet traits. Four treatment combinations were tested: low water velocity (8 cm/s) with 0.5% BW ration, low water velocity with 1.0% BW ration, high water velocity (15 cm/s) with 0.5% BW ration, and high water velocity with 0.5% BW ration. Results from the study are currently being analyzed and will be reported in the next phase of this project. Preliminary results indicate fish fed a 1% diet grew faster than those given a 0.5% diet, with no effect due to water velocity. Despite the decreased rations, both 0.5% ration groups continued to grow throughout the study. Physiological differences among treatments have not been detected with respect to velocity or ration level. Fillet moisture and fat were not affected by feeding rate or water

velocity; nonetheless, there was a tendency for fillets from the 1% BW group to contain more fat than the 0.5% BW group (6.7 v. 6.4%) at day 84 of the study. At low water velocity, 1.0% BW feeding produced a softer fillet than 0.5% BW (587.8 v. 674.0 g). As time on the experiment increased from 28 to 84 days, ultimate fillet pH decreased from 6.7 to 6.5. Feeding rate and water velocity did not affect fillet yield.

Effect of vitamin E supplementation of quality of fresh and smoked trout fillets. The objective of this study is to determine if vitamin E super-supplemented finishing diets improve the quality and extend the shelf life of fresh and smoked fillets. This study will also address how long such finishing diets should be given prior to harvest. Additionally, the effects of fasting fish prior to harvest on the vitamin E content in the fillet will also be investigated. This study is tentatively scheduled to start Spring 2002.

Effect of Aqui-S^{tm} and CO_2 stunning on stress response and early postmortem metabolism in trout fillets. Aqui-S^{tm} Study: Aqui-S^{tm}, a proprietary form of eugenol (the active ingredient in clove oil), has been approved in Australia and New Zealand for use as an anesthetic when harvesting food fish (salmon sp.). It is currently undergoing review by the FDA in the USA. The objective of this study was to determine the anesthetic effectiveness of clove oil when harvesting rainbow trout. Rainbow trout were exposed to the manufacturer's recommended dose of 17 ml/kL, for 15 min in a static water bath. Physiological responses (chloride, cortisol, glucose, and hematocrit) were monitored intermittently throughout the study. Control fish were exposed to an equivalent amount of saline in a static water bath. Preliminary results indicate, while anesthetic treatment was effective in immobilizing the fish, it did not inhibit or prevent a stress response (as indicated by chloride, cortisol, and glucose responses).

CO_2 Stunning Study: The objective is to identify the optimal CO_2 concentration necessary to harvest fish without excessive acidosis or inadequate anesthetization. Three carbon dioxide concentrations were tested (375, 1200, and 2100 mg/L) with control fish stunned (harvested) via a blow to the head.

Objective 4. Technology Transfer. Determine and implement appropriate technology transfer activities to disseminate information generated by this project to the aquaculture industry in Appalachia, to state agencies with aquaculture.

The following activities were completed:

Hosted seminars featuring farmers from Virginia, Prince Edward Island, Canada and researchers from Arkansas, Idaho, Pennsylvania and Ohio State, to describe a variety of aquaculture programs of research, teaching, and business.

Developed bioassays with cooperation of two coal companies at four acid mine drainage treatment plants. Integrate resources and assistance of local economic development authorities, Northeast Regional Aquaculture Center, the Water Resources Research Institute, Mountain Partners, Inc., and WVU.

Responded to over 250 requests (78% increase over 1999) for information from 41 counties, 15 states, and three foreign countries. Conducted site evaluations.

Hosted the Second annual Aquaculture Forum (January 20, 2001) attended by about 100 people, an increase of about thirty percent. Presentations from the Aquaculture Forum are featured at WVU Extension Service Aquaculture web page (http://www.wvu.edu/~agexten/aquaculture/aquacult.htm).

Developed a web site for the Aquaculture Food and Market Development Project. (http://www.caf.wvu.edu/afmdp/)

Publications/Outreach:

Semmens, Kenneth, 2000. "Economic Development and Coldwater Aquaculture in West Virginia" at US Trout Farmers Association, Branson, Missouri, September 2000.

Green Lands Magazine, a publication of the West Virginia Mining Association, Winter 2001, "Somewhere over the Rainbows." 31:1 pp. 26-28

Consol Energy News, "Farming Fish, CONSOL Energy helps WVU to research raising trout in mine water." December 2000. 15:3.

Fidler, Frank, 2000. The Economics of Trout Production in West Virginia, M.S. thesis. WVU.

Miller, Daniel 2000. A GIS Database for Spring and Mine Water Sources in West Virginia, Version 2, January 2001.

Fidler, Frank, 1999. "Where are the Best Counties in West Virginia for Potential Aquaculture Production?" A GIS Analysis. [http://www.nrac.wvu.edu/rm391/fidler/].

Fincham, Ryan, 1999. "Locating Potential Aquaculture Sites in West Virginia Based on Proximity to Demand Outlets." A GIS Analysis [http://www.nrac.wvu.edu/rm391/fincham/].

Roger C. Viadero, and Aislinn Tierney "Technical Feasibility Of Using Treated Mine Waters To Rear *Oncorhynchus Mykiss*", Abstract, Aquaculture America 2002 conference, San Diego, CA, January 27, 2002.

Cunningham, James, and Roger C. Viadero, Effluent Characterization Of Flow-Through Aquaculture Operations In West Virginia, Abstract, Aquaculture America 2002 conference, San Diego, CA, January 27, 2002.

Objective 5. Water Resource Engineering. Characterization of effluents from West Virginia trout production facilities, optimize a working system, and evaluate use of impaired water from mine sites.

5.1 – Baseline quality data and characterization of effluents from West Virginia trout production facilities.

From September to December 2001, the anonymous sites involved in the West Virginia aquaculture effluent study have been visited at approximately six-week intervals.

Field measurements of flow, pH, conductivity, temperature, dissolved oxygen, and turbidity were made of both influent and effluent waters.

Grab samples of influent and effluent waters were also collected and analyzed to determine settleable solids, total suspended solids, 5-day biochemical oxygen demand (BOD_5), nitrogen species (NH_3, NH_4, NO_2, NO_3), and total phosphorus concentrations.

Mass loadings of water quality constituents were calculated using flow and concentration data and compared with current regulatory requirements.

A relationship between turbidity and BOD5 is being studied to ascertain whether turbidity measurements can be used as a low-cost indicator of effluent water quality; however, additional data will be acquired and analyzed prior to recommending this tool for broad use.

5.2 – Technical assessment of impaired water resources suitable for production of trout in West Virginia.

During the reporting period, research was performed to determine the technical feasibility of raising rainbow trout (*Oncorhynchus mykiss*) in treated acid mine waters by:

1) Evaluating the quality of treated acid mine waters relative to those reported in the literature for successful trout rearing.
2) Conducting preliminary bioassays to ensure the viability of fish in the treated mine waters and to assess the bioaccumulation of metals (*e.g.*, mercury, lead, zinc, cadmium) in fish flesh.

An existing treatment facility was selected and surveyed to serve as a research site for the implementation of pilot-scale modular composite material raceways. An agreement for the formal use of water and land resources between WVU and the coal company should be finalized in late August or early September 2001.

Five sample stations were established at the study site to provide baseline water quality data and to evaluate any statistical changes in the quality of waters at the proposed location of the demonstration unit.

1) Water quality monitoring using field meters was conducted on a weekly basis. Parameters measured on a weekly basis included: pH, conductivity, water and air temperatures, turbidity, dissolved oxygen concentration, and water flow rate.
2) Monthly “grab” samples were taken to develop in-depth knowledge of metals and other constituents of the effluent waters. Parameters measured on a monthly basis included: hardness, alkalinity, acidity, sulfate concentration, iron concentration, aluminum concentration, manganese concentration, total suspended solids concentration.

A preliminary field review conducted at MW01 (on Thursday, December 13, 32001) to develop a plan for benthic macroinvertebrate sampling in order to benchmark biological productivity conditions in MW01 receiving waters prior to installing, stocking, and operating the modular raceway system. Full sampling for macroinvertebrates will likely be conducted during the first quarter of 2002.

Site clearing, excavation at MW01 was supervised by WVU-CEE personnel and was completed in October 2001. Final site grading and construction of "footers" will be performed when HFRP raceways are delivered to the site in early Spring 2002.

Objective 6. Farm Level Research. Conduct research at the farm level focusing on production efficiency of facilities growing food size rainbow trout and fish health.

6.1 – Develop and implement a pilot yield verification program for food size rainbow trout in flowing water systems at two commercial facilities.

Preliminary results from yield verification trial at High Appalachian, Inc. shows 50% more weight of fish was raised in tank fed high energy feed (48% protein, 18% fat) compared with standard diet (38% protein, 11% fat). Production capacity in one trial of this mine water based facility ranged from 83 to 138 lb/gpm flow when data is converted on an annual basis. The second trial at High Appalachian is expected to be complete in January of 2002.

The first trial at Trout Lodge and Angler's resort has been completed. Data is being tabulated and analyzed.

6.2 – Health survey of trout production facilities in West Virginia.

A total of fifteen farms were sampled in Year 1. Farms qualified for sampling if they raised salmonids from the egg stage. Six of fifteen farms were privately owned facilities. One qualifying private farm did not participate. Nine of fifteen farms were run by two government agencies. One government agency produced fish at two farms and the other produced fish at seven farms. Farms produced rainbow, brook, brown and golden trout. One farm raised Arctic char.

At least 75% of the production of the private farms was used for stocking/fee fishing. Total annual production (pounds sold) of the private farms is about 250,000 to 300,000 pounds. One government agency raises fish for food, and its total annual production was about 150,000 to 200,000 lbs for 2001. The other government agency raises approximately 700,000 lb of trout each year for stocking.

All farms used spring water that was presumed to be pathogen-free. One farm combined spring water with river water. For three farms, the water collected in a pond or ran above ground before entering the rearing units.

Six of the farms purchased eggs from Trout Lodge, one purchased eggs from Canada, one purchased eggs from Black Canyon in WA, two used only their own brood stock and five had their own brood stock and/or received their eggs from another West Virginia facility.

Fish were sampled according to the requirements of the state of Maryland. A total of sixty fish were sampled from each farm. They were checked for the following viruses, bacteria and protozoa:

- IPNV - Infectious pancreatic necrosis – 1/15 farms positive; fish also showed clinical signs of IPN
- IHNV - Infectious hematopoietic necrosis virus – 0/15 farms positive
- VHSV – Viral hemorrhagic septicemia virus – 0/15 farms positive
- SVCV – Spring viremia of carp virus – 0/15 farms positive
- *Renibacterium salmoninarum* – Bacterial kidney disease (BKD) - 2/15 positive; fish did not show any clinical signs of BKD (note that these results are likely to represent false-positives)
- *Aeromonas salmonicida* – furunculosis - 2/15 positive, fish did not show clinical signs consistent with furunculosis
- *Yersinia ruckeri* - enteric redmouth disease – 0/15 positive
- *Myxobolus cerebralis* – whirling disesase - 8 out of 15 farms were sampled, two were positive. Fish showed signs consistent with whirling disease at both of these farms. (Both of these positive farms raised fish in dirt ponds.)

These results are encouraging and suggest that, at the time of sampling, the prevalence of certifiable pathogens was, as a whole, low in the West Virginia salmonid industry. Production related diseases, such as bacterial gill disease, coldwater disease, protozoal diseases and bacterial diseases secondary to fin erosion often represent the greatest cost to productivity in these systems. As the project contiues year-to-year, Dr. Bebak-Williams will continue to work with these farms to improve the results for the certifiable pathogens and address these "production" diseases.

FY 2000 (Grant 3).

Objective 1. Impaired water research.

Research was conducted to minimize raceway handling and manufacturing costs while improving structural performance and durability through the design, optimization, and implementation of a raceway system constructed of a novel honeycomb fiber-reinforced polymer (HFRP).

Computer aided design of HFRP raceways was conducted in coordination with Dr. Plunkett of KSCI. Cross-sectional and plan views of a modular raceway are presented in Figures 1 and 2, respectively.

Coupon samples of HFRP material were tested in WVU-CEE structural engineering test facilities as part of preliminary tank design.

Preliminary drawings and design features of HFRP raceways have been completed.

Objective 2. Economic Analyses.

2.1 To quantify the economic development impacts from expansion of the aquaculture sector in West Virginia.

As part of this analysis, statewide economic impacts (or multipliers) for: (a) two different levels of food-fish production increases (one- and three-million lbs, respectively), and (b) two types of fee-fishing increases (local and non-local anglers, respectively) were estimated using IMPLAN economic-development software. Results obtained so far show that if aquaculture production in WV doubles from current production levels, statewide output and value-added (which includes additional wages and business tax revenues) would increase by approximately $2 million and $1 million, respectively, and 55 additional jobs would be created.

2.2 To evaluate the impacts, potential for, and consumer acceptance of new production technologies, such as genetically modified, transgenic and organically grown fish, on aquaculture production, prices and profits.

A nationwide telephone survey of 7,500 randomly-selected households will be conducted to address this task. A questionnaire has been developed (draft attached to this report), and the WVU Survey Research Center will be sub-contracted to interview households. The results should be useful to producers and policy makers as they seek ways to meet growing demand for aquaculture products in a manner that is sustainable.

2.3 Economic analysis of impaired water production facilities.

Data are being collected from the ongoing bio-assays at mine sites around the state. These data, together with price data from secondary sources, will be used to conduct the economic assessment of this task.

Other ongoing analyses that do not relate to a specific task but that contribute to the overall objectives of the project include an economic assessment of alternative species and production systems. To this end, development of an enterprise budget for hybrid striped bass in ponds and an enterprise budget for bait fish production are under way.

Work in Progress:

Miller, D., K. Semmens, G. D'Souza, and D. Smith. 2001. "Enterprise Budget for Hybrid Striped Bass in West Virginia Hill Ponds." Agricultural and Resource Economics Working Paper, West Virginia University.

Miller, D., K. Semmens, G. D'Souza, and D. Smith. 2001. "Fathead Minnows as a Supplemental Income Source from Hill Ponds in West Virginia." Agricultural and Resource Economics Working Paper, Division of Resource Management, West Virginia University.

Miller, D., K. Semmens, G. D'Souza, and D. Smith. 2001. "Waste Management in Aquaculture." Agricultural and Resource Economics Working Paper, Division of Resource Management, West Virginia University.

Objective 3. Product Quality

Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. Rainbow trout fillets that had been frozen for 78 d were brined in 8.7 or 17.4% (w/v) sodium chloride solutions, for 30, 60, 90, or 120 min at 3°C. Brine uptake, brined and cooked pH, cook yield, shear force, total and water-phase salt content, and brined and cooked proximate composition were measured on trout fillets. Fish mince was used for texture (hardness and cohesiveness) and protein solubility (total soluble and myofibrillar proteins) evaluations. Increased brine concentration increased fillet weight loss after brining, cook yield, water-phase salt content, shear force, brined fat, brined and cooked ash, brined pH, and brined and cooked moisture. Brining time linearly increased myofibrillar protein solubility, fillet weight loss after brining, total and water-phase salt content, and cooked ash content and linearly decreased water activity and brined and cooked moisture content. A quadratic relationship existed between brining time and shear force, with a maximum force at 90 min. At 8.7% brine, quadratic relationships were observed between hardness and cohesiveness, and brining time with maximum hardness and cohesiveness at 90 min. An 8.7% (w/v) brine concentration and 90-min brining time resulted in maximum texture development while achieving the water-phase salt content ($\geq$ 3.5%) required by the Food and Drug Administration.

Effect of vacuum tumbling with direct salting or brining on smoked trout fillets. Federal regulations require at least 3.5% water-phase salt (WPS) in refrigerated, vacuum-packaged, smoked fish. Evaluations of accepted brining protocols revealed inconsistency in reaching this WPS target. Therefore, other protocols were evaluated for their efficacy in achieving this WPS content. Five brining and direct salting treatments were applied with or without vacuum tumbling: 1) brining, B; 2) direct salting, DS; 3) tumble followed by brining; TB; 4) tumble followed by direct salting, TDS; and 5) tumble with brine, TWB. A randomized complete block design was conducted with three replications. Cook yield, moisture, protein and fat were not affected ($P>0.05$) by treatment; however, shear force of DS (652 g) was greater ($P<0.05$) than TB (480 g). The WPS of DS (3.2%) and TDS (3.3%) was highest, and the lowest WPS was found in TWB trout (1.5%). Although the target was not reached under conditions studied, DS and TDS resulted in product closest to the target, and DS values were most consistent.

Effect of time, sodium tripolyphosphate, and sodium chloride on model trout batters. Ionic strength and pH contribute to protein behavior throughout formulation and processing. Two NaCl levels (2 and 4%), two STPP levels (0 and 0.4%), and 3 holding time were considered. Time did not affect ($P>0.05$) PS of treatments without STPP; however, extending the holding time to 48 h reduced ($P<0.05$) PS. NaCl and STPP did not affect ($P>0.05$) cook yield for trout minces. NaCl did not affect ($P>0.05$) hardness of trout minces; At 0 h, STPP decreased ($P<0.05$) hardness, at 16 h STPP had no affect ($P>0.05$), and at 48 h, STPP increased ($P<0.05$) hardness. STPP did not affect raw pH, free sulfhydryl content, CY, gel hardness and cohesiveness in trout ($P > 0.05$).

Outcomes:

Two abstracts of this work have been submitted for presentation at the 2002 Institute of Food Technologists Annual Meeting and Food Expo.

Objective 4. Technology Transfer.

The following activities were completed:

We hosted of a state wide meeting for supporters of on January 12, 2002 in Flatwoods, West Virginia featuring investigators from each objective describing aquaculture research at WVU. The program also featured producers from Ohio and West Virginia.

Responded to over 300 requests (a 20% increase over 2000) for information from 51 counties, 14 states, and one foreign country. Twenty-nine site evaluations were conducted. Numerous presentations on commercial aquaculture were conducted in response to requests from county agents and other groups.

Demonstrations included the following:

- Bioassay of Arctic Char at a mine water source in McDowell County
- Development of an abandoned Acid Mine Drainage treatment plant as a fee fishing venue.
- Grass carp for the control of aquatic vegetation in a two acre hill pond.
- Induced Spawning of Paddlefish.
- Seining fish at Phil Layne's Farm

"Marketing Arctic Char" was the focus of a road trip to meet seafood buyers at the Food Authority in Jesup Maryland. This trip benefited representatives from four separate West Virginia aquaculture organizations.

Publications/Outreach:

Jittinandana, S. J. 2002. Effect of brine concentration and brining time on quality of smoked rainbow trout fillets. J. Food Sci. *Accepted.*

Mathias, J. S. 2001. Effect of vacuum tumbling with direct salting or brining on smoked trout fillets. MS Thesis Report, West Virginia University, Morgantown. An abstract of this work has been submitted for presentation at the 2002 Institute of Food Technologists Annual Meeting and Food Expo.

Miller, Daniel, Ken Semmens, and Qingyun Sun. Aquaculture as a Post-Mining Land use in West Virginia. World Aquaculture Society meeting, San Diego, CA, January 27, 2002.

Semmens ,Kenneth, Cyril Logar, and Thomas Ponzurick, "Fee Fishing in West Virginia", Appalachian Studies Association Conference at Showshoe Mountain Resort, West Virginia, March 30 2001.

Semmens ,Kenneth, Cyril Logar and Thomas Ponzurick "West Virginia Fee Fishing Survey", Tri-State Meeting of Fisheries Biologists, Huntington, West Virginia. March 8, 2001.

Semmens, Ken, and Rakesh Chandran 2001. Start now to control next year's aquatic weeds. In West Virginia Farm Bureau News, August, 2001.

Semmens, Ken 2001. To learn more about fish, visit Aquaculture Forum, in West Virginia Farm Bureau News, November, 2001.

Wang, Weiqiao, Julio F. Davalos, Roger C. Viadero, Kenneth Semmens, Jerry D. Plunkett, 2001. Fiber-Reinforced Polymer Sandwich Panels for Modular Raceway Fish Culture Tanks. 16th Annual Technical Conference by the American Society for Composites, Blacksburg, VA. September 9-12, 2001.

FY 2001 (Grant 4).

It is too early in this work to report significant progress.