

Project Title: Aquaculture Product and Marketing Development Project.

Principal Investigators: WVU Faculty responsible for specific tasks include:

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Project Duration:

May 1, 2007 through September 1, 2008

Project Description

This project builds upon previous Aquaculture Research at West Virginia University with the Aquaculture Product and Marketing Development Project. It has seven parts intended to focus on specific problems and opportunities faced by growers in West Virginia and the region. They are:

- 1) Enhanced lipid removal during isoelectric protein recovery from Rainbow trout (*Oncorhynchus mykiss*) processing by-products
- 2) Value-added products from aquaculture wastes
- 3) Assessment and development of a legal/institutional framework for the development of the aquaculture industry in West Virginia
- 4) Developing and evaluating trout stocking strategies at Pipestem Resort.
- 5) Role of omega-3 fatty acids and fish consumption in symptoms of reactive airways disease.
- 6) Effect of feed containing *Phaffia rhodoxyma* on the external appearance and flesh color of rainbow trout.
- 7) Aquaculture Extension. This task includes operation of two flowing water facilities in support of aquaculture research and extension.

Justification.

Economic development in West Virginia is critical, particularly in rural communities where traditional economic activities (principally coal and timber) have declined. Rural economic development has been a focus of the *Aquaculture Product and Marketing Development Project*. Strategies where aquaculture can impact economic development in West Virginia and adjacent states are (1) flowing water systems, including the development of mine water resources, and (2) niche markets for WV aquaculture products, including the use of live fish in recreation. Benefits derived from the work proposed in this document have potential for farmers growing fish for both food and recreation. As such the work will encourage development of a dual market for growers in the region strengthening both components of the industry. Work presented in this proposal is designed to complement the work which has been completed and the work which is presently underway.

Specific Objectives

Objective 1. Enhanced lipid removal during isoelectric protein recovery from Rainbow trout (*Oncorhynchus mykiss*) processing by-products.

Problem / Opportunity and Justification

Filleting trout requires removal of bones, skin, head, and viscera (by-products). Mechanical filleting of 100 lbs of trout yields approximately 40 lbs of fillets and 60 lbs of by-products. The 60 lbs of by-products contain approximately 20 lbs of meat and 5 lbs of fish oils (lipids). The by-products are mainly land-filled. Fish processors incur expenditures for the removal of the processing by-products from their plants. The by-products are also an environmental bio-burden.

Funding from previous WVU Aquaculture Food and Marketing Development Project (AFMDP) has allowed development of basic parameters for protein recovery from trout processing by-products. These developments resulted in two patent applications filed by WVU with the U.S. Patent and Trademark Office and several publications.

While these initial developments resulted in protein recovery yield at above 90% (dry basis), the recovered protein typically contains fish lipids at approximately 10% (dry basis). Based on fatty acid profile analysis of lipids in the recovered protein, the lipids contain high content of polyunsaturated fatty acids (PUFA). Although these fatty acids are considered beneficial for human cardiovascular health, they are also highly susceptible to lipid oxidation reaction, which leads to rancidity development. Rancidity of fish food products is associated with a typical fishy odor, which most consumers find offensive. Therefore, the recovered protein as well as the food products in which the protein may be used have a relatively poor storage stability due to rancidity (i.e., fishy odor).

Although 10,000 x g force is normally applied during isoelectric protein recovery, this relatively high g force does not allow separation of fish lipids from the protein, resulting in high retention of lipids in the recovered protein. The PUFA in fish are primarily associated with phospholipids (Figure 1). Due to amphi-philic characteristics of phospholipids (i.e., hydro-phobic and -philic), they interact with both hydrophobic (lipids and hydrophobic proteins) and hydrophilic (water and hydrophilic proteins) compounds (Figure 1). It has been well-established that phospholipids are a major factor contributing to rancidity and offensive fishy odor; and at the same time, phospholipids create a protein-lipid-water emulsion that is difficult to remove. Therefore, the lipids and phospholipids are retained in the recovered trout protein.

It is hypothesized that the enhanced removal of phospholipids from the recovered trout protein will result in greater storage stability of the recovered trout protein as well as the food products in which the protein is used as a functional ingredient.

Objectives

An overall objective is increasing storage stability of the recovered trout protein. The specific objectives are:

- 1) Application of anionic, cationic, and non-ionic food-grade emulsion breakers (also referred to as de-emulsifiers) to enhance lipid removal during protein recovery from trout processing by-products.
- 2) Development of gels from the recovered protein. These gels are typically used to mimic marketable food products.
- 3) Determination of storage stability of the recovered protein and the gels prepared from the recovered protein.

Approach and Procedures

The approach and procedures are aimed to fulfill the specific objectives indicated above: Three types (anionic, cationic, and non-ionic) commercially available emulsion breakers will be used at three different concentrations during solubilization step in the isoelectric protein recovery. The recovered protein, as well as the starting material (i.e., trout processing by-products), will be analyzed for total lipid (Soxhlet extraction, dry basis). The % lipid removal will be determined on a dry matter basis

Analysis.

Moisture (%) and total lipid (%)

To determine % moisture, 2 g of the recovered protein will be placed on an aluminum dish and spread evenly across the dish. The % moisture of the protein will be determined by the oven-drying method (100°C for 18 hrs).

Total lipid content in the recovered protein will be determined according to the Soxhlet extraction method. The sample size will be 5 g and extraction with petroleum ether will

be performed for 16 hrs at a drip rate of 10 ml/min. Total lipid content will be determined on a gravimetric basis and expressed as percent (dry basis).

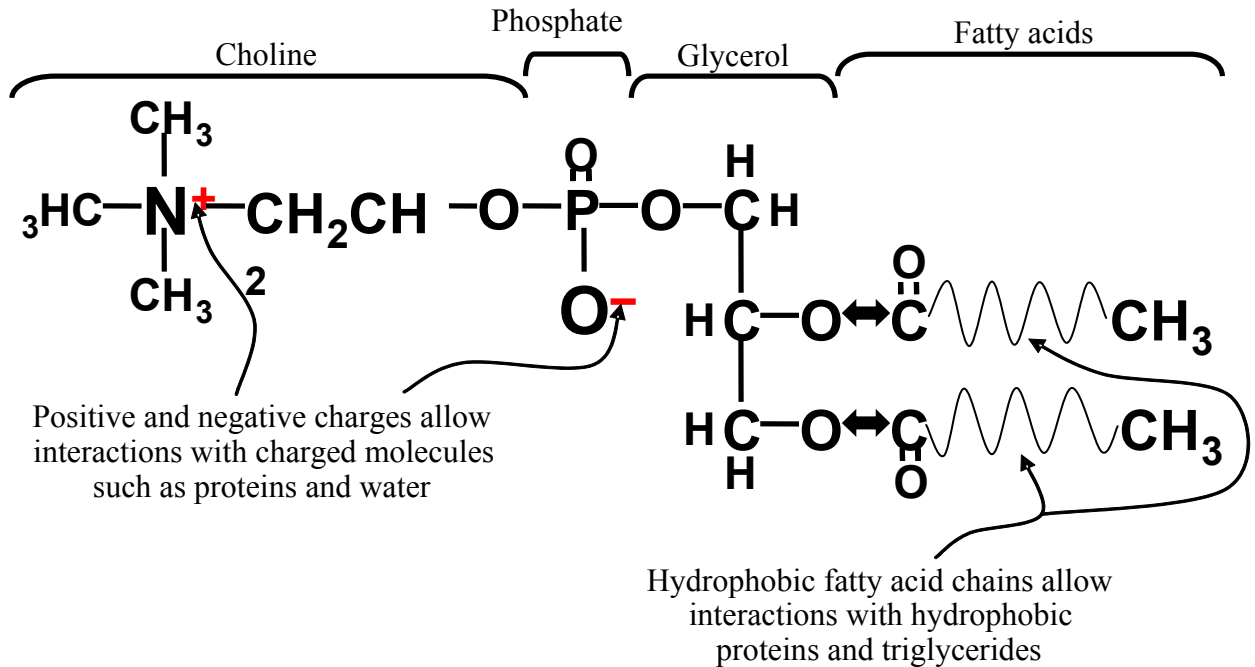


Figure 1. Phospholipids, a major class of meat lipids, are water soluble and interact with charged proteins and water dipoles.

Statistical analysis

The differences between means will be tested using ANOVA at 0.05 probability level. At least six samples ($n = 6$) will be randomly obtained and analyzed. All statistical analyses of data will be performed using SAS (2002).

- 1) The recovered protein will be used to make laboratory gels in order to mimic marketable food products. The recovered protein will be mixed with cryoprotectants (4% D-sorbitol (wt : wt), 4% crystalline trehalose (wt : wt), 0.3% polyphosphate (wt : wt)) and chopped in a universal food processor at low speed for 1 min, resulting in a protein paste. Salt (2 %, wt : wt) will be added and the protein paste will be chopped at low speed for 0.5 min. The functional additives (1% beef plasma protein (BPP) (wt : wt), 2 % exogenous transglutaminase (TGase) (wt : wt), 3% potato starch (PS) (wt : wt), and 0.3% polyphosphate (PP) (wt : wt)) will be added and the final moisture content will be adjusted to 78% by adding ice to the paste, followed by chopping at low speed for 1 min. Additional chopping will be performed at high speed under vacuum (0.5 bar) for the last

3 min. Final pH 7.00 ± 0.05 of the protein paste will be verified with a pH meter and adjusted as needed. During chopping, the temperature of the paste will be controlled between 1-4°C. Following chopping, the paste will be stuffed into stainless steel tubes (length = 17.5 cm, inner diameter = 1.9 cm) and cooked in a water bath set at 90°C for 15 min. The trout protein gels will be chilled in ice slush and removed from the stainless steel tubes. Trout protein gels prepared in this manner will be used for determination of their storage stability.

- 2) The recovered protein and the trout protein gels will be either individually placed in vacuum pouches, labelled and vacuum packed or they will be individually placed on plastic trays and aerobically over-wrapped (non-vacuum) with a typical household plastic wrapping material. The vacuum- and non-vacuum packed samples will be subjected to storage at 2°C. This temperature will be applied to estimate storage stability at the ideal refrigeration conditions in terms of rancidity development and degradation of omega-3 fatty acids. Two storage periods will be selected based on correlation between thiobarbituric-acid-reactive-substances (TBARS, see below) values in the tested samples and rancidity development (i.e., consumer acceptance) reported by Ke, Cervantes, and Robles-Martinez (1984) and by Chen, Nguyen, Semmens, Beamer, and Jaczynski (2007). In addition, an un-trained panel will monitor overall appearance and smell (non-vacuum only) of the tested samples during storage in order to assess when the storage should be stopped and the samples analyzed. The objective will be to analyze recovered protein and the gels when the rancidity development is slightly in progress, but acceptable by an average consumer (first storage period); and more advanced, typically considered as unacceptable by an average consumer (second storage period).

Analysis.

Sample preparation.

Following each storage period, the recovered protein and the gels will be individually homogenized in a laboratory blender (Model 51BL31, Waring Commercial, Torrington, CT) and analyzed for moisture, total lipid, thiobarbituric acid reactive substance (TBARS) values, and fatty acid profiles (FAP) according to Chen, Nguyen, Semmens, Beamer, and Jaczynski (2007).

Moisture (%) and total lipid (%)

The % moisture and % total lipid will be analyzed as described in (1).

Measurement of thiobarbituric acid reactive substances (TBARS)

Oxidative rancidity of fillets will be measured by a 2-thiobarbituric acid reactive substances (TBARS) assay of malondialdehyde (MDA). Three drops of antioxidant (Tenox 6, Eastman Chemical Div., Kingsport, Tenn., U.S.A.) and 3 mL of TBA will be added to 0.2 g of the homogenized sample. Then, 17 mL trichloroacetic acid-HCl reagent will be added. The solution will be flushed with nitrogen and closed. A blank will be prepared in the same manner, but without sample. The tubes will be boiled for 30 min, and then cooled. The colored solution (15 mL) will be centrifuged at $5000 \times g$ for 15 min. A clear and colored supernatant will be transferred to a cuvette, and the

absorbance will be measured at 535 nm using a UV/ Vis spectrophotometer (model DU530, Beckman Instruments, Fullerton, CA). The TBARS value will be calculated based on molar absorptivity of MDA ($156,000 \text{ M}^{-1}\text{cm}^{-1}$ at 535 nm) and the results will be reported as mg MDA/kg of sample.

Lipid extraction and fatty acid profile analysis

The fatty acid profile will be determined only in the recovered protein and the gel samples that retained fish lipids despite application of emulsion breakers applied in objective (1). Lipids will be extracted using methodology described by Folch, Lees, and Sloane (1957) and used for analysis of fatty acid profile. According to the procedure of Fritsche and Johnston (1990), fatty acids will be trans-methylated by the addition of 4 mL of 4% (w/v) methanolic H_2SO_4 and heated in a 90°C water bath for 60 min. The mixture will be saponified by transferring through a Na_2SO_4 filled glass Pasteur pipette and subsequently dried under N_2 in a 60°C water bath for 60 min. The fatty acid methyl esters (FAME) will be re-suspended in filtered isooctane. The FAME and standards will be analyzed by using a gas chromatograph (Varian CP-3800 gas chromatograph, Varian Analytical Instruments, Walnut Creek, CA) and a flame ionization detector fitted with a wall-coated open tubular (WCOT) fused silica capillary column (50 m length, 0.25 mm inside diameter; Varian Analytical Instruments, Walnut Creek, CA). Injection and detection temperature will be maintained at 220°C and column temperature was 190°C . The stationary phase will be CP-Silica 88 (Varian Analytical Instruments, Walnut Creek, CA). Nitrogen will be the carrier gas, and a split ratio of 10 to 1 will be used. The fatty acids will be identified by comparing their retention times with known standards (Sigma, St. Louis, MO) and references (Ackman, 1980). Peak area and the amount of each fatty acid will be computed by an integrator using the Star GC workstation version 6 software (Varian Analytical Instruments, Walnut Creek, CA).

Statistical analysis

The statistical analysis will be conducted as described in (1).

Expected Results and Deliverables. It is expected that some of the food-grade emulsion breakers will result in enhanced removal of fish lipids from the recovered protein. Therefore, application of emulsion breakers during protein solubilization used to recover muscle protein from trout processing by-products may be necessary. The removal of fish lipids will likely result in greater storage stability and consumer acceptance of the recovered trout protein as well as the trout protein gels (i.e., marketable food products).

The investigator has already initiated collaboration with two fish processors. Mr. Luis Marmol owns the largest rainbow trout farm/processing plant in West Virginia (formerly Trout Lodge and Angler's Resort). Carp Protein Products (South Carolina) has signed a non-disclosure agreement with WVU and is in process of licensing the WVU-patent pending technology to recover muscle proteins from carp species. The investigator has also continued research collaboration with WV Aqua. The research findings generated from this proposed project will be directly applied to the benefit of the above companies. Therefore, this proposed project will contribute to both state and out-of-state fish growers/processors.

Manuscripts for publications in peer-review journals will be prepared and submitted. The data from this proposed research will also be used for posters/oral presentations at national level professional conferences.

In addition, the research conducted based on funding from previous cycles of the AFMDP project has resulted in collaboration with other WVU faculty. For example, the investigator collaborates with Dr. Moritz to determine digestibility of the recovered trout protein for applications in animal feeds; with Dr. Tou to determine the quality and safety of the recovered protein for human food applications; and with Dr. Matak to apply continuous bio-reactor system for algae growth. These spin-off projects have resulted in various publications, attracted external support, and allowed students including undergraduate students to conduct scientific projects.

Personnel:

Dr. Jacek Jaczynski

Institutional Units Involved:

Animal and Nutritional Sciences

Objective 2. Value-Added Products from Aquaculture Wastes

Justification:

Aquaponics, the simultaneous culture of fish (aquaculture) and plants (hydroponics), has the potential to provide an additional income source for growers through the sale of a luxury foodstuff as well as reduce nutrient export from fish production. There are many advantages of aquaponic plant production (human consumables or ornamentals) including: utilizing the nutrient rich water from aquaculture that otherwise would have been a waste product or would need to be filtered in a costly manner, eliminating the cost and time involved with mixing traditional hydroponic nutrients, providing an organic natural form of nutrients for the plants, reducing soil borne diseases by eliminating the soil in vegetable production. Plant spacing can be very intensive, allowing one to grow more plants in a given space, and crop production can occur in an aquaponic system year-round. Aquaponics has been developed in conjunction with recirculating aquaculture systems (RAS). Limited aquaponics research has been done utilizing flow-through aquaculture production systems. Research conducted at WVU could be utilized in other locations that use a flow-through aquaculture system. The aquaponics system located at the Reymann Memorial Farm (RMF), a unit of the West Virginia Agriculture and Forestry Experiment Station (WVAFES). can also be used to compare production capabilities with other aquaculture systems including recirculating and pond production.

Significance to West Virginia. Horticultural crop production is one of the fastest growing agricultural markets in the U.S. and West Virginia. This includes food and ornamental (landscaping and cut flowers) production. One of the major limiting factors in horticulture production is consistency. Retailers want to purchase a product from a grower on a consistent basis. Utilizing this aquaponics system, growers not only can

produce a consistent product for sale to local and regional retailers but they will be able to grow salad greens during off seasons allowing production when the traditionally grown crop is scarcer (winter and summer) thus increasing profit margins for a grower. Using the current aquaponics system available at the Reymann Memorial Farm, a grower has the potential for a yearly minimum profitability of \$20,000 to \$40,000 just on the salad greens depending on the harvesting schedule used. This profit is substantial for a small fish producer in West Virginia. Increased horticulture production utilizing the fish effluent should provide a means for reducing nutrient discharge into the major U.S. watersheds. This will also provide a means for fish farmers to stay within the EPA regulations for nutrient management. This system as compared to standard aquaculture or horticulture practices requires very little maintenance to produce a value-added commodity. This is ideal for a small fish grower to increase income with minimal additional labor especially in off-season fish producing periods.

Previous Work and Present Outlook:

Previous support of aquaponics research over the last 2 years has yielded answers to many research questions.

1. Demonstrated that aquaponics can be achieved in a flow-through system. All previous work has been done in recirculating systems.
2. Demonstrated that water temperature is an important factor for plant growth and development. Demonstrated that cool season plants (food and ornamental) grow well in this system; warm season plants do not. This establishes the importance of water temperature in determining which plants to put in the system. Cool season plant trials (food consumables and ornamentals) are ongoing for 2007 to determine other key plants that will perform well in this cool water system.
3. Plant production can be maintained year-round providing a reliable income source. Additionally, the system can be used to grow cool season crops through the summer when they are less available and can command a higher price.
4. Nutrient recovery was low. Upper limits of percent removal were 20% of ammonia, 7% of nitrate and 4% of phosphate.
5. Nutrient recovery was greatest during summer when light availability and air temperatures were greatest.

Publications from this work include:

Derek J. Dyer, 2006. Effectiveness of aquatic phytoremediation of nutrients via watercress (*Nasturtium officinale*), basil (*Ocimum basilicum*), dill (*Anethum graveolens*) and lettuce (*Lactuca sativa*) from effluent of a flow-through aquaculture operation. M.S. Thesis, West Virginia University, Morgantown, WV.

Erika Nichole Smith, 2007 Watercress (*Nasturtium officinale*) Production Utilizing Brook Trout (*Salvelinus fontinalis*) Flow-through Aquaculture Effluent. M.S. Thesis, West Virginia University, Morgantown, WV.

Smith, N. , T.P. West, K.M. Buzby, R.C. Viadero and K.J. Semmens. Evaluation of Watercress (*Nasturtium officinale*) as a Phytoremediation Option for Brook Trout (*Salvelinus fontinalis*) Aquaculture Effluent. Presented at Aquaculture America, Las Vegas, NM 2006.

K. M. Buzby, D. Dyer, R. C. Viadero, Jr., T. P. West and K. J. Semmens. Evaluation of the nutrient removal capabilities of watercress, lettuce and basil from a flow-through aquaculture system. Presented at Aquaculture America, San Antonio, TX, 2007.

D. J. Dyer, K. M. Buzby, R. C. Viadero, Jr. , T. P. West, and K. J. Semmens. Effectiveness of aquatic phytoremediation of nutrients via watercress (*Nasturtium officinale*) from effluent of a flow-through aquaculture operation. Presented at Aquaculture America, San Antonio, TX, 2007.

Current Research:

Current research will examine the impact of periodic harvests on plant growth and nutrient removal. It is expected that both production and nutrient removal will be improved with this production system as the root system, where nutrient uptake takes place, remains intact. Support in the current year is necessary to maintain support staff that are integral to the project.

Compared to recirculating systems, the system at Reymann Memorial Farm produces less plant biomass and is less effective at nutrient removal.

Comparison of Flow-through System vs. Recirculating System

	Plant Biomass (g/m ² /day)	Nitrate % Removal	Phosphate % Removal
Flow-through System (Reymann Memorial Farm)	4-7	7	4
Recirculating System (Adler et al. (2003))	12	30	99

It may be possible to increase production after determining what factors are limiting production. Limited production may be due to nutrient limitation. Nutrient concentrations at RMF were as much as 5x lower than other flow-through systems in WV. This nutrient limitation for plant growth may directly impact uptake of nutrients from the fish effluent. By adding additional essential nutrients such as nitrogen, the plants may have increased biomass production resulting in increased nutrient uptake. Low water temperatures may also inhibit nutrient uptake from the fish effluent. Optimum growth of many plants occurs with root zone temperatures between 22 & 35 °C (Bode Stolzhus et al. 1998) while water temperatures at RMF are 15.5 °C. There may be

insufficient contact time for the roots to effectively take up the nutrients. Light availability may be insufficient especially during the winter. Identification of which factors are most important in nutrient uptake limitation and consequently limit production is critical to maximizing production from flow-through aquaponics systems.

Currently, production consists of floating rafts to support the plants. These rafts contain vermiculite (plant growing medium) as the growing substrate for the plants. As plant growing medium is an extra cost to the grower, a more sustainable substitute would be desirable. One such replacement could be developed from the fish solids that are produced from the aquaculture system. These fish solids could be composted and then used to replace the vermiculite which would eliminate the extra production cost of purchasing the necessary plant growing medium. This compost could also be used as an amendment to standard plant growing media as an organic fertilizer source which may increase the production capabilities and provide a more controlled fish solids management program. This composting system would provide a more environmentally sound and sustainable method of solids management with the added benefit of value-added use.

Funding for this Aquaponics Research Facility has provided the opportunity for two competitive funded grants. One grant from the Northeast Sustainable Agriculture Research and Education (NESARE) is to evaluate ornamentals utilizing effluent from a flow-through aquaculture system and another is from the Northeastern Regional Aquaculture Center (NRAC) to evaluate aquaponic plant production (plants used for mitigation). Both grants are multi-state participating projects.

Collaborative efforts with WVU Extension Service are being developed to provide a grower demonstration and production scale-up in the immediate future. This will provide West Virginia fish producers the opportunity to see what they are capable of doing and how to develop their own aquaponics production system. The proposed objectives of this project are essential in providing the necessary data for advancing to the next phase of grower demonstrations and recommendations for flow-through aquaculture/aquaponic systems of varying capacities.

Objectives:

1. Enhancement of aquaponic production

To determine which factors limit nutrient uptake and biomass production within a flow-through aquaponics system and to provide information for growers with varying production capacities and input levels.

2. Fish Waste Solids Management and Horticultural Utilization

To investigate the feasibility of using vermicomposting to compost fish waste solids as an amendment to standard plant growing media for production of market value added products of ornamental and aquaponic plant production.

Procedure:

Studies will be conducted at the WVU Division of Plant and Soil Science Agronomy Farm and Greenhouse located in Morgantown, WV, WVU Aquaponics Facility at the Reymann Memorial Farm in Wardensville, WV and at Dogwood Lake, a WVAFES flow-through aquaculture facility at near Morgantown, WV.

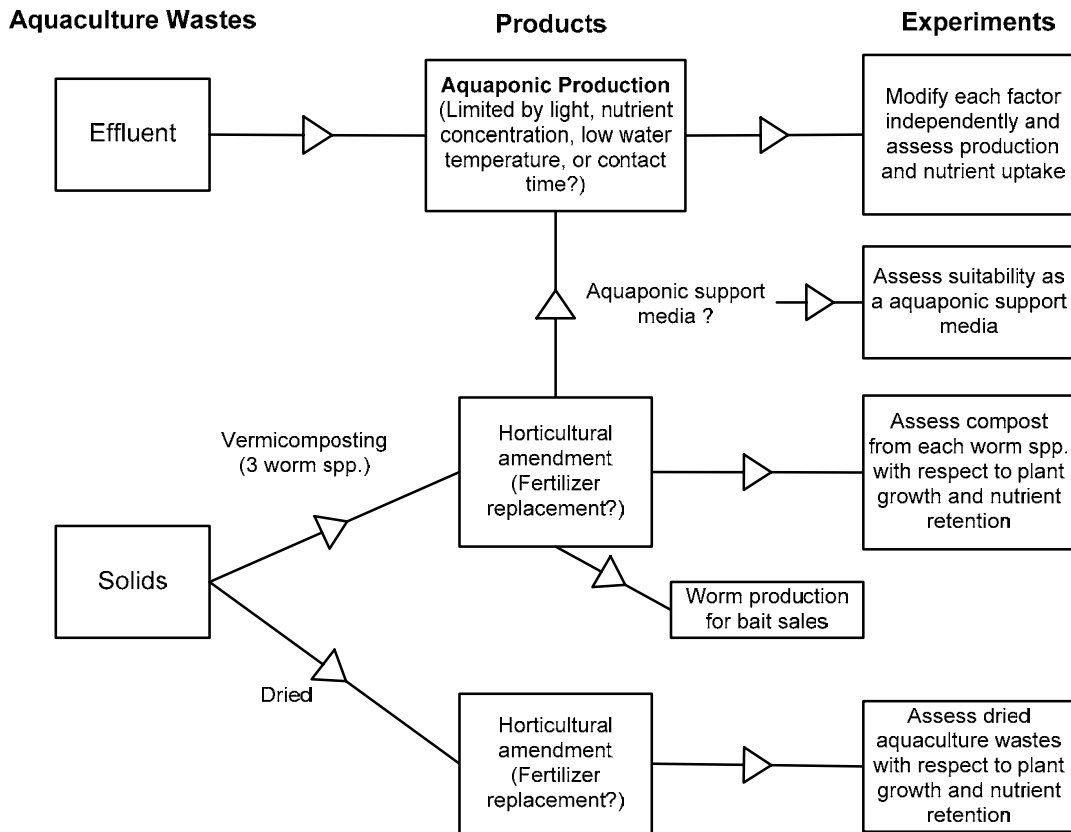


Figure 2. Overview of value-added products resulting from aquaculture wastes in conjunction with data needs and experiments that will address those data needs.

Enhancement of aquaponic production. Nutrient concentration, water temperature, light availability, and contact time will be examined as potential factors limiting aquaponic production within a flow-through aquaponic system. Nutrient concentrations will be increased by dripping supplementary nutrients from a carboy mounted at the top of the channel. Target values will approximate a large flow-through production facility and a more median value. With 3 nutrient concentrations (ambient, medium and high) it will be possible to understand how production and nutrient uptake vary with nutrient concentration and consequently be able to advise growers whose nutrient concentrations vary from our system. Water temperature will be elevated via a solar water heating system. Although using a solar heating system may produce more variable results, the

inputs required by a water heater to elevate temperatures was deemed excessive and certainly not cost effective for most growers. Water temperatures in the channels will be monitored by a HOBO Tidbit temperature logger. Light availability will be increased by mounting high output lights (controlled by a timer) above the channels. Photoperiod and intensity will approximate summer values. A datalogger will be used to monitor light and temperature. This experiment will be conducted in the fish building at RMF due to security issues as well as to avoid light contamination on the rest of the experiment. Contact time will be increased in 2 ways; increased channel length or reduced water depth.

Each factor will be evaluated independently with 3 replicates. Experiments will be conducted using watercress and 1 additional plant species. The additional species will be determined from current plant trials at the Aquaponics Research Facility in Wardensville. Plants will be started from seed to ensure uniform starting conditions as well as to provide continuity with previous experiments. Plant biomass (fresh weight, dry weight, shoot length, root length) and water quality (NH_3 , NO_2 , NO_3 , PO_4) will be monitored every 3 weeks. Monitoring will be initiated 6 weeks after the experiments begin; previous experiments have determined that prior to 6 weeks plants are too small to take biomass measurements as well as too small to remove measurable nutrient quantities. In addition, a cost/labor analysis will be conducted to determine if additional inputs produce sufficient yields to be cost effective.

Fish Waste Solids Management and Horticultural Utilization. Aquaculture is a growing industry within agriculture for West Virginia. Another rapidly growing economically important agricultural commodity in West Virginia is the production of ornamental plants (nursery production for the landscaping industry) and food consumables such as salad greens (watercress and lettuce) and small fruits (strawberries and raspberries). The food and nursery industry could benefit from the aquaculture industry directly by reducing input costs by using composted fish solids as an organic fertilizer amendment to standard plant growing media. Having an output for fish solids would also benefit fish growers as well by providing them an environmentally sound method of fish solid waste disposal with value added production. This project could assist in increasing funding possibilities by providing new opportunities for nutrient studies on nursery and aquaponically grown crops.

Solid wastes (fish feces and uneaten food) are generated in aquaculture facilities and require removal. Allowing solid wastes to remain in the raceway system reduces oxygen availability due to particle decomposition, increases nutrient release as the particles decompose, and increases disease risk. Quantities of waste solids generated can be substantial; Boardman et al. (1998) estimated that 7.86 kg/day were generated in an experimental raceway. Once removed from the system they constitute a waste product that must be disposed of. However, rather than a waste, the solids might be considered a resource. Composted fish solids, when mixed with a planting growing medium; represent both an organic matter and nutrient source. Hinshaw and Fornshell (2002) report that fish wastes were 8.1% nitrogen (N) and 4.3% phosphorus (P).

Efforts to recapture dissolved or suspended nutrients from farm effluents have included the production of other economically important aquatic species such as agar-producing algae and mussels, diverting nutrient-rich effluents into secondary crayfish or baitfish ponds to produce an additional crop. Wetlands have been used as 'biological sponges' to remove nutrients from water by slowing the water which allows solids to settle out and wetland vegetation to absorb nutrients. Recently (2006), Virginia Tech researchers received a USDA grant to examine the viability of fish solids management utilizing vermicomposting to produce compost for use in ornamental plant production. Vermicomposting is defined as the practice of using concentrations of earthworms to convert organic materials into usable compost (worm castings). The worms used for composting provide a management method to convert fish solids to a usable horticultural organic plant growing medium amendment. Fish farmers utilizing aquaponics need some type of plant substrate to grow the plants on and vermicomposting may provide a sustainable alternative to purchasing high cost commercially available products. This use of vermicompost is highly desirable because fish do not have an indigenous bacterial flora such as *Escherichia coli* which is only found in mammals (Geldreich and Clarke, 1966). *E. coli* can only be present in an aquaculture system through feed or environmental contamination (Del Rio-Rodriguez et al., 1997). Use of vermicompost over other composted materials such as livestock or poultry manure will prevent human disease pathogens such as bacteria and liver flukes from being introduced into the aquaponic production system which is of great concern in U.S. agriculture production systems.

Earthworms species used in vermicomposting are also typically used as a fishing bait worms. Vermicomposting will reduce the discharge of nutrients into the watershed by removing high nutrient containing solids from the system while providing the farmer with a sustainable source of a plant growing media amendment as well as increasing profits through bait sales. Worms produced can be sold locally or regionally at retail outlets that sell live bait. Typically, aquaculture production facilities are located in natural fishing areas which would have a demand for live bait (worm). Management of fish solids utilizing vermicomposting is not limited to flow-through aquaculture production systems; this also has the potential for use in other production systems such as recirculating or ponds.

Solids will be collected from the flow-through raceway facility at Dogwood Lake. The solids will be collected as the quiescent zone drains exit above ground where they will be piped into an open-topped mini-geotube via gravity feed. The solids will be allowed to settle, and the overlying clear water siphoned off. At each collection the solids will be characterized (pH, EC, %C, %N, %P, sulfate concentration, and particle size distribution). The fish solids will be composted utilizing vermicomposting.

Three common composting worms will be evaluated for production of vermicompost which include European Night Crawlers (*Eisenia hortensis*), Redworms (*Eisenia fetida*) and Georgia jumper (*Amyntas gracilis*). These three worms were chosen based on their basic biology. The European Night Crawler (ENC) is a large worm species well suited for use as a bait worm and its ideal temperature range is a bit cooler than is that of

Redworms and it requires higher moisture levels than other worm species used in vermicomposting (Edwards and Bohlen, 1996). This may be very significant with composting fish solids. ENC are considered to be the least desirable species of those used for vermicomposting because of their low reproductive and growth rates. Redworms are the worm species identified as the most useful in vermicomposting systems and are the easiest to grow in high-density culture because they tolerate the widest range of environmental conditions and fluctuations, and handling and disruption to their environment of all species identified for this purpose (Edwards and Bohlen, 1996). Redworms are a small worm (1-2” in length) and not always suited for use as bait. Georgia Jumpers are also a large worm species well suited for use as bait. Georgia Jumpers are a tropical species with a poor tolerance for cold temperatures which will affect their productivity in vermicomposting in colder seasons.

Composting Worm Comparison

Worm Type	Temperature Range	Worm Size	Reproductive Rate	Use for Vermicompost	Use as Bait
European Night Crawlers	55° F-65° F	Large	Low	Only for cold wet systems	Well Suited
Redworms	70° F-80° F	Small	Moderate	Highly desirable	Not well suited
Georgia jumper	70° F-80° F	Large	Moderate	Moderately desirable	Well suited

Based on the biology of the worms, each of these three worms will produce composts with different characteristics and one may be more suitable for composting fish solids. The three types of compost produced by the three different worms will be characterized (pH, EC, %C, %N, %P, sulfate concentration, and particle size distribution) and will be evaluated as a sustainable organic media supplement or amendment for horticultural production.

This study will consist of two components. One component will evaluate the vermicompost as a replacement for vermiculite (plant growing medium) used in the current WVU Aquaponic Research Facility. This study will be arranged in a 2x4 factorial random block design evaluating watercress and lettuce production and vermicompost type (European Night Crawler, Redworm, Georgia jumper and Control – Vermiculite). The second component will evaluate vermicompost and non-composted dried fish solids as an organic amendment for containerized plant production. Vermicompost and non-composted dried fish solids will be incorporated into plant potting media at rates of 0, 25, 50 and 75% compost/medium volume. This study will be arranged in a 3x4 factorial random block design evaluating compost type (4 types; 3 vermicomposts and 1 non-composted fish solids) and incorporation percentage (25, 50, and 75) and will include conventional fertilizer and no-addition controls. The plant that will be grown will be a pot mum (garden mum)(*Chrysanthemum ×morifolium*) and was chosen because it has a summer growing season and is salable for fall. Plants will be evaluated for growth including height and two-directional width, plant nutrient levels will be also measured (total nitrogen, total phosphorus), and biomass production (fresh and dry weights). Garden mum sales could potentially give fish farmers more

diversification to provide more income to their production facility. In addition, a cost/labor analysis will be conducted to determine cost effectiveness.

Personnel:

Dr. Todd P. West, Assistant Professor of Horticulture
Division of Plant and Soil Sciences

Dr. Karen M. Buzby, Postdoctoral Fellow
Department of Civil and Environmental Engineering

Dr. Todd P. West will oversee the horticulture component including maintaining the plants, data collecting including measuring plant biomass and nutrient analysis. Dr. Karen M. Buzby will be responsible for maintaining and modifying the Aquaponics Research Facility in Wardensville, designing and implementing the solids capture at Dogwood Lakes, data collecting including nutrient analysis (water quality, and ion resin bags), solids and compost characterization and datalogger data.

Institutional Units Involved:

Division of Plant and Soil Sciences.
Division of Animal and Nutritional Sciences.
Department of Civil and Environmental Engineering.

Objective 3. Assessment and development of a legal/institutional, framework for the development of the aquaculture industry in West Virginia

Justification:

Aquaculture cannot exist or expand without a clear understanding of the legal parameters under which it must exist and flourish. In order to conduct aquaculture operations in the light of the concept of adequate risk management (legal), in order to secure capital investments for expanding or commencing operations and in order to promulgate policies which might impact statutory and regulatory activity, one must have a clear understanding of the current regulatory/statutory schemes and the agencies charged with implementing and enforcing these provisions. And, in order to promulgate proposed changes to the current aquaculture laws in West Virginia, one must look to other jurisdictions for guidance in how to restructure or revamp our current laws. Finally, in order to impart the information discerned from this research, one must develop a course of study for both current growers and future growers (students). In order to maximize the accessibility of this course of study, the materials should be made available in both the classroom and remote (online setting).

The benefits of this course of education and research are obvious. Because aquaculture operations are faced with not only financial challenges but legal ones as well, a course of research focusing on compliance with laws will assure that the grower not only enters the industry with full knowledge but understands current ongoing legal and/or risk management issues with a degree of competency that will assure the growth of the

operation. Additionally, this course of research will aid in the ultimate goal, that being a comprehensive set of legislative/regulatory recommendations that are necessary to make West Virginia competitive in the aquaculture industry.

If aquaculture is expected to grow in West Virginia, it is simply unacceptable to continue addressing legal issues in a piecemeal fashion and on a case-by-case basis. Statutes and regulations should be comprehensive, easily understood and straightforward in their application. There is a need to determine what agencies are best equipped to promulgate these laws and then implement and enforce them. To determine this, it is necessary to first learn what is not working here in West Virginia and then determine what has worked in other jurisdictions.

Present Outlook:

Previous research relating to the status of law relative to aquaculture in West Virginia has revealed that:

- Statutes and regulations are dispersed among and between various statutory and regulatory schemes;
- These statutes and regulations are not comprehensive in nature;
- These statutes and regulations are sometimes inconsistent with one another;
- These statutes and regulations are not always implemented and/or enforced by the agency most capable of doing so; and,
- These statutes and regulations in their substance do not encourage the growth of aquaculture in West Virginia.

These laws, while purporting to adequately regulate the aquaculture industry, reflect a backward attitude toward the industry and, in fact, discourage capital investments in aquaculture. Further, previous research, while admittedly sparse in this area, indicates that other states have statutory and regulatory schemes that encourage the aquaculture industry. These schemes appear to differ from that of West Virginia in significant ways. For example, a number of the more progressive states clearly qualify aquaculture as agriculture and place the primary control of the industry within the grasp of said state's agricultural agency.

As previous research has progressed, the investigators have come to recognize that if recommendations are ever to be made relative to changing the regulatory/statutory structure in West Virginia, guidance will have to come from those more progressive states.

Additionally, as previous research has been ongoing, it has become readily apparent that fish growers are woefully uneducated on aquaculture law in West Virginia. Having interviewed numerous growers and participated in one aquaculture forum, it has become obvious that growers are in need of specific educational opportunities when it comes to the law that applies to aquaculture.

Previous Work:

1. AQUACULTURE AND THE LAW: AN UPDATE ON WEST VIRGINIA ISSUES
(PowerPoint Presentation at a meeting of APMDP, November 10, 2006)

2. Interviews with fish growers

a. Guyses Run, Fairmont, West Virginia\

DATE: May, 2006

PRESENT: Mike Nardello

b. Whispering Pines Lake, Alum Creek, WV

DATE: May 24, 2006

PRESENT: Brenda and Bub Anderson, Owners

Fred Hayes,

DEPT. AG. REP

c. Freshwater Institute, Shepherdstown, West Virginia\

DATE: June 22, 2006

PRESENT: Joe Hankins. Fish Biologist

d. Craig Yohn, Jefferson County Extension Agent

DATE: June 22, 2006

e. West Virginia Aqua

DATE: July 18, 2006

PRESENT: Greg Ellis and John Browning

3. Other interviews:

a. Ken Semmens

b. Charles McElwee, J.D.

c. Jeff Bowers, Department of Natural Resources

d. George Cottle, Fee Fishing Operation

e. Frank Jernejcic, Fisheries Management, Department of Natural Resources

f. Brett Preston, Fisheries Management, Department of Natural Resources

4. This investigator has prepared a yet untitled body of work which includes both the statutory and/or regulatory reference and comments prepared by the investigator summarizing the law and highlighting problems contained therein. This research encompasses the following areas:

1. West Virginia Water Pollution Control Act (WV Code 22-11-et seq)

2. Solid Waste Management Rule (WV CSR 33-01) (West Virginia Code 22C-3-et seq)(West Virginia Code 22-15- et seq)

3. Sewage Sludge Management Rule (WV CSR 33-02) (West Virginia Code 22-15-20)

4. Standards for Beneficial Use of Materials Similar to Sewage Sludge (WV CSR 33-08) (West Virginia Code 22-15-22(b))

5. Hazardous Waste Management Rule (WV CSR 33-20)
 6. National Pollutant Discharge Elimination System (NPDES) Program (WV CSR 47-10) (West Virginia Code 20-5A-3)
 7. WV Aquaculture Farm Rules (WV CSR 61-23)
 8. WV Fish Processing Rules (WV CSR 61-23A)
 9. Sewage Treatment and Collection System Design Standards (WV CSR 64-47)
 10. Commercial Sale of Wildlife (WV CSR 58-63)
 11. Miscellaneous Permits and Licenses (WV CSR 58-64) (Sales and Ponds: West Virginia Code 20-2-48) (Commercial Fishing West Virginia Code 20-2-53)
 12. Catching & Selling Bait Fish (WV CSR 58-62) (West Virginia Code 20-2-55)
 13. Animal Disease Control (WV CSR 61-01)
 14. Certified Pesticide Applicator Rules (WV CSR 61-12A)
 15. Fish Importation Permit (WV Code 20-2-13)
 16. Special Rules (Office of Water Resources) (Industrial waste) (WV CSR 47-11)
 17. General Groundwater Protection Rules for Pesticides (WV CSR 61-12G)
 18. Water Well Permit – Sewage System Permit (West Virginia Code Chapter 16) (WV CSR 64-9, 64-47, 64-19, 64-3)
 19. Fishing Regulations (WV CSR 58-60)
 20. Release of Fish and Stocking Permit (West Virginia Code 20-2-64)
 21. License to Take Fish for Commercial Purposes (West Virginia Code 20-2-59)
 22. Permit to Take Fish for Scientific Purposes (West Virginia Code 20-2-50)
 23. Status of Aquaculture Globally
 24. The Clean Water Act, 33 U.S. Code 1251
 25. Effluent Limitations Guidelines and New Source Performance Standards for Concentrated Aquatic Animal Production, Point Source Category 40 CFR Part 451
 26. The National Aquaculture Act of 1980 16 USC 2801
 27. The National Aquaculture Improvement Act of 1985 16 USC 2802
 28. The United States Joint Subcommittee on Aquaculture
 29. State Statutory Schemes for Aquaculture -- An Overview
 30. Ownership of Beds and Banks of Streams in West Virginia
5. Research reviewed:
- a. Aquaculture Operations- Laws, Regulations, and Policies- U.S. EPA
 - b. Maintaining Instream Flow And Protecting Aquatic Habitat: Promise And Perils On The Path To Regulated Riparianism- Lee Breckenridge, Northeastern University School of Law
 - c. National Aquaculture Development Plan of 2000- Joint Subcommittee On Aquaculture National Science And Technology Council
 - d. Public Ownership Of Rivers In The U.S.- National Rivers Organization
 - e. Navigability Primer- Jason Robertson
 - f. Ownership Of Beds And Banks Of Streams In West Virginia- Charles McElwee, J.D.
 - g. River Law: Fact and Fiction- National Rivers Organization
 - h. Real Property: Rural Landowners' Liability And Posting of Land- Thomas Allen, Dennis K. Smith, Anthony Ferrise

- i. West Virginia Fishing Regulations Summary 2006- West Virginia Division of Natural Resources
- j. Waste Management In Aquaculture- Dan Miller, Ken Semmens
- k. Mining Freshwater For Aquaculture- Appalachia Magazine, Carl Hoffman
- l. State Environmental Laws Affecting West Virginia Agriculture- National Association of the State Departments of Agriculture Research Foundation
- m. Nonindigenous Aquatic Nuisance Prevention And Control Act of 1990- 16 U.S.C. Sections 4701-4751
- n. Attack Of The Alien Invaders- Frank Jernejcic
- o. Aquaculture Outlook- David J. Harvey
- p. National Aquaculture Legislation Overview- Food And Agriculture Organization
- q. Compliance Guide For The Concentrated Aquatic Animal Production Point Source Category- U.S. EPA, Federal Register: 40 CFR Part 451
- r. State Policies For Aquaculture Effluents And Solid Wastes In The Northeast Region- John W. Ewart, Joe Hankins, Dan Bullock
- s. Effluent Guidelines: Aquatic Animal Production Industry- U.S. EPA
- t. Water Rights And Water Quality: Interrelationships And Implications- Janice M. Schneider, Paul Singarella
- u. Annual Report Fiscal Year 2004-2005- West Virginia Department of Environmental Protection
- v. Public Rights in West Virginia Watercourses: A Unique Legacy of Virginia Common Lands and the Jus Publicum of the English Crown, 101 W.Va. Law Review 407 (1998)- Larry George, J.D.
- w. Water Rights and Riparian Rights, W.Va. State Bar CLE (1997)- Larry George, J.D.

6. West Virginia Aquaculture Forum: This investigator attended this forum on January 20, 2007. Using the above-referenced research, the investigator moderated a meeting of representatives from the West Virginia Attorney General's Office, The Department of Natural Resources, The West Virginia Department of Environmental Protection, The West Virginia Department of Agriculture and the West Virginia Public Land Corporation and the attendees of the forum. Attendees of the forum were given the opportunity to inquire of the representatives and this investigator rephrased the questions if necessary, directed said question to the appropriate representative and then summarized the representative's response in vernacular easily understood by the questioner.

7. This investigator has participated in various gatherings wherein legal issues pertaining to aquaculture have been addressed. For example, this investigator has met with and advised those working on the development of recreational fishing opportunities at Pipestem.

Objectives:

1. Statutory and regulatory schemes in the more progressive aquaculture states will be studied in detail, both for structure and substance. Using this material, the investigator

will prepare a document entitled “Regulatory Schemes for Aquaculture: Observations and Recommendations”. This document would clearly outline the various regulatory schemes used by states with developed or developing aquaculture industries. These schemes would then be compared and contrasted with the current structure used by West Virginia and recommendations would be made as to feasible changes. This document would be written in such a manner as to be easily deciphered by regulators, legislators, farmers and/or industry experts.

2. In order to further educate growers, educational materials will be developed which will focus on the various agencies whose actions impact aquaculture, water rights as they pertain to aquaculture, business operation issues related to aquaculture and environmental issues as they relate to aquaculture.

Procedures:

In order to gain necessary information dealing with specific laws in other jurisdiction, in-depth research will be conducted similar to that that has been completed for West Virginia. Then, these regulatory and/or statutory schemes will be compared and contrasted to that of West Virginia for the purpose of determining what specific changes should be made in West Virginia. A number of states have developed aquaculture plans. These states provide a rich field for study. Potential states to be studied and the year of their plans are:

**Arkansas (1991),
Georgia (1996)
West Virginia (1999)
Indiana (1997)
Pennsylvania (2003)
Florida (2005-06)
Missouri (1998)
Massachusetts (1995)
Indiana (1989)
Wisconsin (1990)
Louisiana (1992)
Florida (2001)
North Carolina (1988)
New York (1993)
New Jersey (1995)
Idaho (date), Maine (1980)
Hawaii (1979)
Kentucky
Ohio (2001)
Massachusetts (1995)**

Upon completion of the legal research referenced above, the investigator will recommend a proposed aquaculture plan outline which plan outline will serve as the basis for specific statutory and regulatory changes relative to aquaculture in West Virginia.

Personnel:

This research will continue under the direction of Fonda L. Holehouse, a principal investigator in the previous research. She is a visiting assistant professor currently teaching the following courses at the Davis College of Agriculture, Forestry and Consumer Sciences at West Virginia University: Agriculture and Natural Resource Law, Environmental Regulation, Enterprise Operation Law, Current Issues in Agriculture and Rural Enterprise Development. Sara Brown, a GRA in the DCAFS whose previous experience in legal/policy research will be used to aid in the research process.

Institutional Units Involved:

Division of Resource Economics.

Objective 4. Developing and Evaluating Trout Stocking Strategies at Pipestem State Park, WV**Justification:**

West Virginia Division of Tourism and Natural Resources developed a 5 year strategic plan (2003-2007), which emphasized the importance of hunting and fishing in the state as a way to increase the impact of nature-based tourism on the West Virginia economy. Despite the demand for fishing packages in West Virginia by community leaders and tourists alike, there continues to be a lack of fishing opportunities available in the state. Poor catch-rates on public waters continues to be among the reasons reported for this scarcity.

Heavy fishing pressure on public water (due to population growth and urban expansion) and unequal fishing effort at highly accessible stocking locations (due to truck following) result in declining average fish sizes and catch rates (Radomski, Grant, Jacobson, & Cook, 2001)—both can have a negative impact on angler satisfaction. For example, a typical goal for trout catch rates on public waters can range between 0.25 trout/angler-hour (Watt & Persons, 1988) to 0.7 trout/angler-hour (Miko, Schramm, Arey, Dennis, & Mathews, 1995), yet a mean catch rate considered to be successful by those anglers is often much higher (e.g., 1.44 trout/angler-hour) (Miko et al., 1995). In a study conducted by Pierskalla, Moldovanyi, and Schuett (2003), results indicated that the ideal catch rate on private waters in West Virginia is 5 trout per daily visit.

Finn and Loomis (2001) also reported that the importance attached to catch motives increases as success in catching a preferred fish decreases. The numbers of anglers that experience catch deprivation may be on the increase considering the growing fishing pressure placed on a fixed supply of public waters (Aiken, 1999). Given these findings, it is not surprising that WV anglers are seeking enhanced fishing opportunities.

One approach to dealing with declining catch rates has been to allow non-governmental organizations to supplement governmental efforts in the provision of more stocked fish (Rosenberger, Collins, & Svetlik, 2005). The results of their study indicate that anglers are generally supportive of the program. However, stocking

higher numbers of fish does not always result in higher angler catch rates, unless access to the area is controlled (Butler & Borgeson, 1965). Based on these findings, it seems likely that a similar business model could work for public parks interested in improving angler satisfaction through controlled access.

Pipestem State Park is an ideal test market that has the potential to control access and attract anglers that seek satisfying catch rates. The purpose of this case study is to implement and evaluate several new fish stocking strategies at Pipestem State Park. The study will document the biological and economic feasibility of stocking strategies on the resort, assess the relationship between stocking levels and catch rates, and determine visitors' willingness to pay for future opportunities such as additional fishing packages.

Previous Work:

Previous work had focused on demand for fishery tourism packages by providers and tourists, as well as the potential for such packages at Pipestem. This work (discussed below) will lay the foundation for the currently proposed studies.

Demand for Tourism Packages by Tourism Providers—The advantages associated with developing travel packages has been documented in the tourism literature. Wilson, Fesenmaier, Fesenmaier and Es (2001) conducted a study to identify and examine factors that have helped rural communities successfully develop tourism and entrepreneurial opportunities. They applied a focus group methodology to obtain in-depth information describing community leaders' and business persons' attitudes and perceptions of the process of tourism development. The focus group results suggested that the most important factors for successful tourism development in rural areas involve the development and promotion of the complete tourism package, good community leadership, etc.

Demand for Tourism Packages by Families--Current trends suggest an increase in demand for travel packages by families. Increased work demand of two-income families, in part, has contributed to less leisure time. That is, multiple income families have the money to participate in more expensive recreation activities but have less time to spend together (Douglass, 2000). Fishing packages make family travel more convenient allowing families to have eventful and high quality experiences in shorter periods of time.

Demand for West Virginia Fishing Packages by Tourists--There appears to be substantial demand for fishing packages in West Virginia. Logar, Mei, Pierskalla and Semmens (2003) conducted a survey of potential WV tourists. They found that only 9 percent ($n = 496$) of visitors participated in a travel package to West Virginia. However, 74 percent of them would not rule out participating in recreational package in West Virginia. The situation is that most visitors prefer to participate in a travel package but they have had few experiences in West Virginia. In addition, from their survey, 49 percent of respondents desire fishing or fishing lessons as part of a package. Most respondents travel as a family unit (72%) and desire overnight accommodation as part of the package (85%). Based on their findings, the family fishing package would meet the needs of a significant market segment in WV.

Potential for Fishing Packages at Pipestem State Park --In a study conducted by Mei, Pierskalla, and Schuett (2006), the researchers concluded that family fishing packages

are the most promising tourism package for the Pipestem test market, and perhaps can attract those visitors with the greatest household incomes. Swimming, kayaking, backpack camping, watching wildlife, sightseeing, and target shooting are examples of secondary activities that can compliment family fishing opportunities and were among the more heavily marketed activities near Pipestem. Their results also suggest that the family tourism packages could target those potential tourist between the ages of 30-39, traveling with children (married or single), and with graduate degree education. These results compliment those findings presented by Logar and others (2003).

Demand for improved fishing opportunities at Pipestem State Park—A baseline angler survey was conducted in April (2007) using an on-site interview instrument (Appendix A). A total of 19 anglers over age 18 were contacted at Long Branch Lake during two days shortly after the DNR stocked the lake. The information will be used as a baseline to evaluate the impact of the proposed Fall (2007) stocking initiative. In addition, information will be gathered to enhance future fish stocking programs that offer improved catch rates (number of fish per hour of effort) and catch patterns (variety of fishes caught).

Present Outlook:

Supply of West Virginia Fishing Packages--Limited opportunities for participating in fishing packages exist in West Virginia despite the expressed needs by potential tourists. Additional development of fishing packages through public-private partnerships can help attract visitors from a larger region if those packages afford higher quality and more eventful experience opportunities. For example, higher catch rates and more recreational activities can contribute to more eventful fishing packages. Better customer service and larger fish sizes can contribute to improved quality. However, for each of those production factors, several constraints exist (e.g., heavy fishing pressure on public water, unequal fishing effort at highly accessible stocking locations, declining average fish sizes and catch rates, and lack of marketing) in the market creating difficult challenges for individual tourism providers (Finn & Lommis 2001; Radomski, Grant, Jacobson, & Cook, 2001). These challenges will be addressed in this study.

Present Outlook for the Aquaculture Industry as it Relates to Recreation--A key factor constraining aquaculture industry development in WV is lack of information relating to economics (i.e., production costs and revenues), market development, and risk management. While new products and production methods are often associated with the potential for higher revenues and/or lower production costs, their adoption is constrained by lack of producers' technical knowledge and expertise.

On the demand side, the recreational market, in particular, appears poised for rapid growth. Thus, selection of stocking strategies and fishing packages to exploit this demand and stimulate interest in WV's aquaculture-related recreational opportunities requires better understanding of such factors as the target market, package pricing, seasonal factors, and the income contribution of new, fishing-oriented packages to existing amenities such as golfing or hunting at the location (e.g., state park or resort) of interest.

This research will build upon research conducted in previous grants, with the goal of providing the industry with the information needed to establish a new business or facilitate growth of existing operations. Economic information is one of the many components of an interdisciplinary, systems analysis that includes biological, recreational, business, extension and other components, the complementarities of which are essential for sustainable development of the industry.

Objectives:

This proposed study will identify and evaluate trout stocking strategies for the Bluestone River, Long Branch Lake, and Par 3 pond. The study objectives for each of the three study locations follow.

- (1) Bluestone River—to determine the impacts of alternative stocking strategies offered at the resort on: (a) the resort itself (e.g., influence room occupancy and visitor expenditures in the park) and (b) the guest (e.g., angler satisfaction, demand for fishing opportunities, and willingness to pay for additional recreation opportunities), and to investigate trout stocking and harvesting strategies designed to enhance recreation and fishing opportunities for guests and the local community;
- (2) Long Branch Lake—to investigate trout stocking and harvesting strategies designed to enhance recreation and fishing opportunities for guests and the local community; and

Procedure:

Pipestem State Park is the test market for our proposed case study. The park is located 14 miles north of Princeton and 12 miles south of Hinton in southeastern West Virginia. Because of the abundant tourism resources at or near Pipestem Resort State Park (Bluestone River, Long Branch Lake, several small ponds, guest lodges, rental cottages, campgrounds, golf courses, gourmet restaurants, modern conference facilities, swimming pools, ball courts, and horseback riding), the radius of 30 miles was set as the test market boundary. The test market was limited to the state of West Virginia for this initial study (Figure 1).

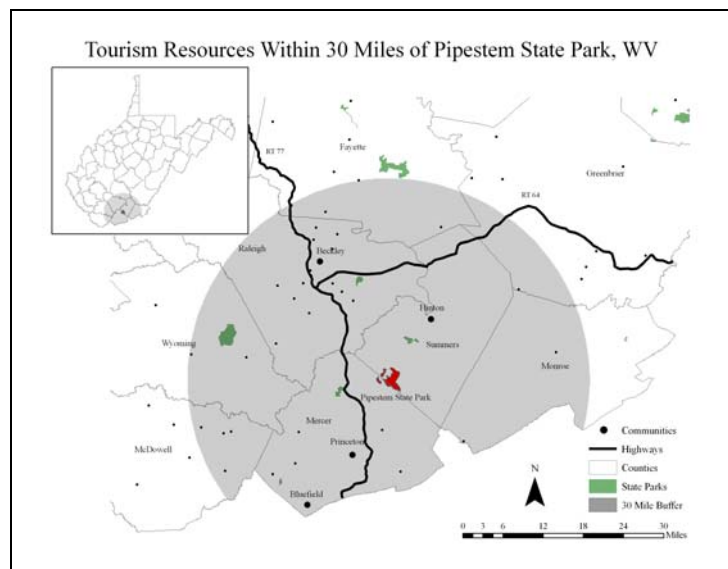


Figure 3: Tourism resources within 30 miles of Pipestem Resort State Park

The Bluestone River at Pipestem State Park represents an excellent opportunity to study the practicality of using cultured trout in public waters as a means of enhancing local economies and tourism, specifically targeting out of state anglers and families. The river is not easily accessible to local anglers seeking to “use” fish stocked for our purposes, but it is easily accessible for stocking. The Bluestone River at Pipestem is accessible via tram or a steep, strenuous 4 mile hike from the state park to the river valley on one side of the river. Private property and the state park lodge flanks the other side of the river. No current trout fishery exists in the river so no conflicts with “historical” fishing uses would be expected by our proposal to stock trout in this reach to benefit aquaculture and tourism in the state.

Several questions emerged regarding the development and evaluation of trout stocking in the water resources at Pipestem State Park. These questions and associated research procedures are classified as: (1) biological; (2) recreation; and (3) resource economics.

(1) Biological

The Bluestone River at Pipestem does not presently contain trout, at least in part because of its location above Bluestone Lake and because the state does not stock there presently. Because trout are not already present we need to learn more about how stocked trout behave and how they return to the creel in support of our recreation and resource economic studies. Some of the questions we have are: Do stocked fish move outside of the stocked areas? Do stocked fish move outside of the area in response to high/low flows? Do they move well outside of the area? How many do we need to stock to achieve target catch rates? From a thermal perspective what is our limit for trout season in the Bluestone River? Also, do different species move or perform better than others? Do different sizes perform differently (re: movement and catch rates)? Will fish stocked in fall overwinter and be available in the spring at significant rates? To address these questions we propose a two pronged adaptive study.

Experiment 1--We propose to stock fish into the study reach of the Bluestone River during Fall 2007 to address these questions. We will stock 700 rainbow trout (purchased from George Cottle) at 1-2 lbs in size, including a small number of larger fish to increase angler satisfaction. Each fish will be fitted with a uniquely numbered anchor tag to monitor movement and recapture locations and rates. Fish will be stocked into a series of about 6 pools in the study reach. Fish will be tagged in batches with different colored tags for each stocking location to permit immediate detection of fish movement even if anglers incorrectly report fish tag numbers.

In addition to external tags we will also implant 20 fish from this stocking with radio tags to monitor short-term movement of fish in response to thermal and flow changes and to help us determine where fish are at under different conditions. A minimum of 20 fish will be radio-tagged in order to assure a sufficient sample size to permit us to publish the results of this study in a scientific journal. Typical radio telemetry studies with fish use results of 12-25 individuals (Bjorgo, 2006). We propose to tag 20 fish in this initial stocking to ensure we will have 15 fish to monitor after allowing for post surgery

mortality and long distance movements of fish outside the study area (Logan, 2003). In addition to learning fish locations relative to river conditions, location information will also assist us in guiding anglers to the fish as part of the stocking evaluation. We will also install water temperature loggers at 3 locations within the study reach to obtain annual thermal conditions in the river to assess the seasonal extent of our possible fish stocking season in the Bluestone River. By stocking in fall with tagged and telemetered fish we will be able to address not only if the fish move outside the study reach, but also whether fish overwinter in the river and become available the following spring.

Experiment 2- Adaptive study--Depending upon the results of this initial experiment we will propose additional experiments in late winter and spring, 2008. If fish show stocking site fidelity we can run experiments to look at angler satisfaction and catch per unit effort, or evaluate angler satisfaction with varied species combinations in different pools. We can also evaluate catch rate relationships with stocking density of different trout species and time – fish size – catch per unit effort interactions.

During spring of 2008 we will initiate stockings with a mix of trout species and sizes so we can evaluate site fidelity, angler capture rates and angler satisfaction. We will stock rainbow trout, brown trout, and brook trout in this stocking with each fish receiving a unique numbered anchor tag for analysis of movements and to permit possible tournament activities in support of the fishing packages. In addition we will again radio tag up to 15 individuals of each trout species to evaluate their movements relative to short-term changes in flow and temperature. Cooler water temperatures during the spring stocking should yield better survival rates of fish following surgical implantation of radio tags, so slightly fewer tags are required in this experiment. Examining movement and habitat use of the three trout species simultaneously will yield a second journal publication in addition to any across-discipline papers that result. Simultaneous tracking of all three species will permit us to see how each species responds under the same environmental conditions in the river and allow us to fine tune stocking recommendations for future use in privatized fishing packages in the Bluestone River at Pipestem and as a starting point in other possible packages at other West Virginia State Parks.

(2) Recreation

What is the standard of quality for various catch-related factors (e.g., catch per unit effort, catch pattern, and catch size) associated with a fishing experience? What stocking levels are required to achieve ideal catch rates? And, how does enhanced fishing experience impact the economic benefits realized by Pipestem State Park? This phase of the case study will examine perceived satisfaction levels to identify indicators and standards of quality for various fishing experiences on the Bluestone River, Long Branch Lake, and par 3 pond. Models of visitor satisfaction including situational variables (e.g., resource, social, and management settings) in a recreational environment and subjective evaluation (e.g., individual characteristics and experiences) of the angler will be developed.

The sampling frame includes all adult guests of both upper and lower resorts during the spring and fall stocking seasons. All adults will be contacted during 8-12 weekdays and 4-6 weekends during both seasons. Each study participant will be given a short on-site

interview (e.g., visitor and trip characteristics, visitor motives, and travel cost variables) and a fishing scorecard (in the format of a diary instrument) for each day of their visit (e.g., profile of catch, catch location, and perceived satisfaction). Visitors will be asked to report the unique tag number found on each fish caught during their trip as well as the location and satisfaction of their catch. Initial contacts with guests will be made during check-in. Study participants will be asked to return the scorecards at the end of their visit to compete for prizes. For example, if any fish identification number matches a predetermined winning number, the guest will win a prize upon confirmation of the entire scorecard. Approximately 300-400 anglers will be contacted during both Fall and Spring seasons.

Assuming the percentage catch-return and angling pressure remain the same during the study periods, catch rates should be related to fish stocking levels. We will determine the factors related to catch rate by using the following formula provided by Moring (1985):

$$Q=(S \times R)/P + W/P$$

where:

Q = catch per unit of effort,
S = number of trout stocked,
R = percentage return/100,
P = angling pressure,
W = number of fish in catch.

The economic impact of the stocking initiative will be estimated based on daily expenditures reported in the diary instruments and based on room occupancy. These findings will be compared with travel cost models to better determine the demand for fishing opportunities at the park.

(3) Resource Economics

We will work collaboratively with the marketing and recreation investigators to examine: (a) the target market for Pipestem State Park fishing sites; (b) the importance of specific attributes of the recreational experience (such as fish size, catch rate, and species); (c) attributes of the location in anglers' decisions to visit the Park (e.g., accessibility, travel time, and presence of other amenities such as golfing or hunting); and (d) the marginal contribution of a fishing package to existing packages offered at the resort. We will also explore how growth in fishing-related recreation opportunities can contribute to industry growth and local economic development.

To address these research questions, we will conduct a contingent valuation (CV) study of anglers visiting Bluestone River, Long Branch Lake, and par 3 pond. The contingent valuation (CV) study will be conducted to estimate anglers' willingness to pay (WTP) for improvement of fishing experiences, specifically: (a) increase in catch rate; and (b) increase in the variety of fish sizes (mix of 1 and 2 lb fish). CV involves the use of surveys to elicit respondents' maximum willingness-to-pay (WTP) for improvements in public goods or services that are not traded in the market. An individual's WTP is equivalent to the enhanced well being that a respondent receives from providing specific

improvements in goods or services (Mitchell & Carson, 1995). CV allows evaluation of a change in the provision of a non-market good prior to initiating the proposed change (Dalton et al., 1998). Contingent valuation has been widely used in the economic valuation of recreational fishers' WTP for improvements in fishing experience. For example, Fields et al. (2004) examined anglers' WTP for the opportunity to fish for new specie – hybrid bluegill. The study was conducted at three West Virginia pay fishing businesses. The result shows that anglers are willing to pay about \$30 per 10 fish for catch-and-keep, or \$29 per 1 hour for catch-and-release fishing opportunities are similar. Rosenberger et al. (2005) examined anglers' WTP for a trout stocking program for Cheat River, WV. The authors found that WTP increases with respondent dissatisfaction with last year's fishing experience, importance of catching a lot of fish, keeping fish after catching them, and the respondent's age, education, and income level. WTP declined among respondents who were not supportive of the stocking program and resided further from the stocking site. Estimated average per-trip mean and median WTP were \$4.60 and \$3.36, respectively. Cantrell et al. (2004) estimated anglers' WTP for increased catch rate resulting from a stock enhancement program for Pacific threadfin in Hawaii. Recreational anglers were willing to pay an average \$7.95 to maintain their current catch level of 3.8 Pacific threadfin per fishing trip. They were willing to pay an even higher amount of \$10.05, \$13.67, \$19.95, or \$20.52 to increase their average catch by 1, 3, 9, or 11 Pacific threadfin per fishing trip respectively. The authors found that employment status, occupation, and income were the variables that affected individual WTP at statistically significant levels. Similarly, Johnson and Walsh (1987) used CV to estimate the value of fish caught at Colorado's Blue Mesa Reservoir, and reported the value of additional trout or coho salmon caught at the site to approximately equal \$0.95. Johnson and Walsh (1989) examined anglers' WTP on the Pourdre River and found that the mean catch was four fish per angler day and the value of catching an additional fish was approximately \$0.78.

We will conduct CV study through in-person interviews with anglers visiting Pipestem State Park (see the description of the sampling frame in "*Recreation*" section above). The interview questionnaire will be based on the survey conducted in April of 2007, and questionnaires used in other related studies. Focus group studies and a pre-test of the interview questionnaire will be conducted to modify unclear or ambiguous questions. Similar to other studies (e.g., Rosenberger et al., 2005; Borisova et al., 2007), respondents' maximum WTP for catch-and-release of one or more additional 1 or 2 lb trout will be elicited using a modified payment card approach (see question 13 of the survey in Appendix A). Such an approach results in WTP responses that are grouped on the intervals defined by the values provided on the payment card. In other words, respondents' WTP values are between the value selected on the payment card and the next higher value. To estimate exact WTP of the respondents, empirical models of WTP as a function of explanatory variables (e.g., respondents' socio-economic characteristics, their use and knowledge of the fishing site, and their attitudes and opinions of the fishing site). Similar to other CV studies, tobit or grouped tobit model will be used to examine WTP (Benson, 2006; Borisova *et al.*, 2007; Cameron and Huppert, 1989; Rosenberger *et al.*, 2005). The model will be estimated using LIMDEP or SAS statistical software.

The information generated from the analysis will be used to help develop stocking strategies for trout that offer the greatest economic gain.

Expected Outcomes. If the study findings suggest that the stocking strategies (or some modification of the strategies) are biological and economic feasible, the park will serve as an important demonstration area and case study that could contribute to the implementation of Senator Helmick's Fish Stocking Initiative, the development of new fishing packages in the park and surrounding area, and enhancement of fishing opportunities for locals and guests of the park. Recommendations will be made regarding the development of new policies and products favoring guests staying at the resort. New ways to encourage day visitors to pay for stream access will also be considered. The findings will be presented at workshops, professional association meetings and similar forums, and will be published in refereed outlets.

Personnel:

Kyle J. Hartman. Dr. Hartman will have primary responsibility for the "biological" studies of stocked fish into the Bluestone River. He will coordinate the evaluation of movement, retention, and return to creel of stocked fish. Dr. Hartman has conducted studies employing tagging and telemetry of stocked fishes in the Ohio River and in WV headwater streams, as well as telemetry studies of wild fish in the Kanawha River West Virginia. This biological data will feed into the economic studies in measures of stocking profitability given biological constraints.

Roy Ramthun. Dr. Ramthun will help administer undergraduate student research for the project. He has a background in outdoor recreation and tourism.

Tatiana Borisova. Dr. Borisova will have primary responsibility for economic analysis of target markets for Pipestem State Park fishing sites and the importance of specific attributes of the recreational fishing experience. She will collaborate with the other specialists on the team, assisting in design of the interview questionnaire and analysis of interview responses for *Resource Economics* and *Recreation* parts of the project. She has research experience in aquaculture economics and natural resource management.

Chad D. Pierskalla. Dr. Pierskalla will coordinate the study of catch related indicators and standards of quality for all stocking strategies implemented in this case study. He will work with the research team to develop instruments, supervise the visitor data collection process, and contribute to report writing and publication. Dr. Pierskalla has research experience in the study of outdoor recreation behavior and human dimensions of natural resources management.

Kenneth J. Semmens. Dr. Semmens, is State Extension Specialist for Aquaculture and specializes in aquaculture with twenty years of experience producing and marketing a wide variety of warm, cool and coldwater fish species. He holds a joint appointment with the Cooperative Extension Service and the West Virginia Agricultural and Forestry Experiment Station.

Institutional Units Involved:

The following units at West Virginia University will contribute to the project: Cooperative Extension Service; West Virginia Agricultural and Forestry Experiment Station; Wildlife and Fisheries Resources; Recreation, Parks and Tourism Resources Program; and Agriculture and Resource Economics. Additional consultation will be provided by the Department of Recreation and Tourism Management at Concord University.

Cooperation:

To help begin the process of developing this proposal, two workshops were conducted in spring, 2005. The preliminary sites examined in this study include Stonewall Resort State Park in northern West Virginia and Pipestem Resort State Park in southern West Virginia. Workshop attendances included county Extension agents in adjacent counties, persons from CVB (Conference and Visitors Bureau) and EDA (Economic Development Administration) in adjacent counties, resort managers and civic leaders in adjacent communities. After identifying existing and potential resources for fee-fishing packages associated with Pipestem Resort State Park and Stonewall Resort State Park and their surrounding locations, Pipestem Resort State Park was selected as the test market for additional gap analysis given its potential for the development of fishing packages. It was the belief of the researchers that this test market would provide the best opportunities for families, and would better address the needs of potential tourists identified in earlier studies. The gap analysis later confirmed those assumptions to be correct.

In March, 2007, WVU researchers met with the West Virginia Division of Tourism to discuss ways to market fishing packages that are developed at Pipestem. A meeting was held a few weeks later with the superintendent of Pipestem State Park and a local fish producer (George Cottle) to discuss the potential for enhancing fishing experience opportunities at the park. The group agreed that Pipestem State Park is a suitable site for future package development and research.

Objective 5. Role of omega-3 fatty acids and fish consumption in symptoms of reactive airways disease**Justification:**

Asthma is a common, chronic disease that affects both adults and children. In 2004, 104 per 1000 U.S. children aged 5 to 17 years had been diagnosed with asthma (American Lung Association, 2006). It affects 11% of West Virginia children. Asthma is defined as a chronic inflammatory disease of the airways. Although many effective therapeutic pharmaceutical agents have been developed, asthma continues to result in high rates of morbidity and mortality, and there is a great deal of interest in alternative therapies as sole or adjuvant treatments. Diet is one such alternative that is thought to play a role in the etiology of asthma as well as in the management of symptoms. Much of the interest in dietary manipulation has been in altering the ratio between pro-inflammatory omega-6 fatty acids and anti-inflammatory omega-3 fatty acids, particularly the marine-derived long chain fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In epidemiological studies, higher dietary intake of fish has been associated with a

decreased risk of asthma in children (Hodge et al., 1996, Tabak et al., 2006). Whether that is because of the omega-3 fatty acids or some other feature of the fish is not well defined. Clinical trials of omega-3 fatty acid supplements have been inconclusive (Reisman et al., 2006). Encouraging increased consumption of fish such as WV trout may have health benefits as well as economic benefits to the aquaculture industry.

The *long-term goal* of this investigator is to understand the relationships among dietary intake, health, and development of infants, children, and adolescents. The *objective of this proposal*, which is a step in pursuit of that goal, is to compare respiratory symptoms of asthmatic children who eat at least one serving of fish per week and maintain a diet high in omega-3 fatty acids with those of children who eat their usual diet. The *central hypothesis of this application* is that a diet that is high in omega-3 fatty acids and includes regular fish intake will decrease symptoms related to airway hyper reactivity. This hypothesis was formulated after a critical review of the literature. The *rationale* for undertaking the proposed work is that asthma is a chronic disease that creates a burden for the families that live with it as well as for the public health sector. Increased intake of fish and other food sources of omega-3 fatty acids are healthy lifestyle changes that may favorably impact airway inflammation and reactivity and will cause no harm.

Previous Work and Present Outlook:

Asthma is a disease in which the airways are irreversibly inflamed and are hyper reactive to allergens in the environment. This hyper responsiveness leads to increased mucus production with coughing, wheezing, and shortness of breath. It is a chronic condition that affects 22 million people in the US (Centers for Disease Control, 2007). The US Department of Health and Human Services has included reducing asthma-related deaths, hospitalizations, activity limitations, and lost school days in its objectives for Healthy People 2010 (US Department of Health and Human Services). Some have postulated that the increased prevalence of asthma during the last 50 years is due to changes in dietary patterns, particularly decreased intake of fruits and vegetables (sources of antioxidant vitamins) and fish (source of omega-3 fatty acids) (Seaton et al., 1994).

Airway inflammation involves a variety of cell types including mast cells, alveolar macrophages, neutrophils, eosinophils, lymphocytes, platelets, and a variety of inflammatory mediators (Rolin et al., 2006). These cells release more than 50 different inflammatory mediators that are responsible for the symptoms and pathology of asthma. The hypersensitivity reaction is thought to be due to an exaggerated response by T-lymphocytes that leads to the release of leukotrienes from activated inflammatory cells. Leukotrienes are derived from arachidonic acid, an omega-6 fatty acid, via the 5-lipoxygenase enzyme pathway. When these leukotrienes bind to receptors they recruit neutrophils, and induce bronchoconstriction and mucus production. They are the targets of several effective asthma medications (Wong, 2005). However, medications have side effects and prolonged use may result in decreased effectiveness. Alternative therapies that increase the effectiveness or reduce the dose requirements of medications would be useful.

Omega-3 fatty acids are thought to have a protective role in the development of inflammatory diseases. Fatty acid composition of cell membranes reflects dietary fat intake. Omega-3 fatty acids compete with and displace omega-6 fatty acids on cell membranes in airways and other substrates. Saturation of cell membranes with omega-3 fatty acids is significantly increased after 2 weeks of supplementation and may reach a peak after 6 weeks (Simopoulos, 2002). Omega-3 fatty acids may suppress the synthesis of arachidonic acid by competitive inhibition of the elongation and desaturation of linoleic acid (omega-6 fatty acid that is a precursor to arachidonic acid). The presence of eicosapentaenoic acid (EPA), a 20-carbon, omega-3 fatty acid inhibits the production of arachidonic acid by negative feedback of the rate-limiting enzyme, thereby decreasing the direct precursor for the production of leukotrienes. EPA competes with arachidonic acid as substrate for the 5-lipoxygenase enzyme, which is a key regulatory enzyme in the leukotriene pathway. Instead of yielding leukotrienes from the four series, which are potent chemoattractants and pro-inflammatory, metabolism of omega-3 fatty acids yields five-series leukotrienes, which are weaker chemoattractants and less inflammatory. Thus increasing the amount of omega-3 fatty acids relative to omega-6 fatty acids may be expected to alter the fatty acid composition of the membranes lining the airways in a way that decreases the inflammatory response to allergens.

Clinical trials that have investigated the effects of omega-3 fatty acid supplementation have been inconclusive. Mickleborough (2005) reviewed the existing data concerning the relationship between omega-3 fatty acids and airway hyperresponsiveness and concluded that there may be a protective effect on airway function in asthmatics. Reisman et al. (2006) reviewed randomized controlled trials for effects of treatment of asthma with omega-3 fatty acids. They found little evidence for clinical effectiveness and consistent reports of adverse effects, such as nausea and abdominal discomfort, which were dose dependent. They suggested that a food-based approach may be better tolerated should the efficacy of omega-3 fatty acids be proven. A food based approach may be one in which the regular intake of fatty fish is encouraged.

Epidemiological data suggest that intake of fatty fish may help to protect against asthma in children. Hodge et al. (1996) reported that children who ate fresh, oily fish once a week were 75% less likely to have current asthma. Eating fish more than once a week was associated with decreased risk for asthma in Australian children (Peat et al., 1992). The intake of fish was inversely associated with asthma in a study of Dutch school children (Tabak et al., 2006). These studies suggest that intake of fish may protect children from developing asthma. Only one study examined the effects of dietary manipulation on symptoms in children with current asthma (Hodge et al., 1998). That study used a supplement of omega-3 fatty acids in addition to changing the diet to decrease the intake of omega-6 fatty acids and asking the participants to eat fish more than once a month. They found no difference in asthma severity scores after six months. More frequent fish intake may be important. A diet that includes adequate amounts of fish and maintains a balance between omega-6 and omega-3 fatty acids is a strategy that will not cause any harm and may have beneficial effects in the prevention of chronic diseases as well as in the management of airway hyper reactivity.

Objectives:

1. Promote fish consumption among children with physician-diagnosed asthma by providing omega-3 enhanced trout.
2. Determine quantitative dietary intake of fatty acids and other nutrients
3. Compare respiratory tract symptoms and medication requirement between children who were randomly assigned to a diet high in omega-3 fatty acids vs. those receiving usual care.

Procedure:

Children with current asthma between 5 and 18 years of age and who receive care for their asthma through the WVU Robert C. Byrd Health Sciences Center will be recruited for this study. The investigator, who has an adjunct appoint with the WVU School of Medicine, Department of Pediatrics will work with faculty from that department to identify and recruit participants. The investigator has received all appropriate training for conducting studies with human subjects as mandated by the WVU Institutional Review Board for the Protection of Human Subjects and will apply for full board approval for the project.

Children and their families will be recruited through the asthma clinic. Written information about the study will be available in the clinic with contact information for the investigators. The nurses and physicians will be asked to help identify potential participants. In addition, the investigator and graduate student will attend to clinic to meet with children and their families to promote the study. Those who agree to participate will be oriented to the study methods. Parents will be asked to give their signed informed consent for their child to participate and the children will be asked to give assent. Children will be randomly assigned to usual care or high omega-3 fatty acid intake including one serving per week of omega-3 enhanced trout provided by the WVU Aquaculture Program. Before beginning the study, the families in the high omega-3 group will be given the opportunity to sample the omega-3 enhanced trout for flavor and acceptability and will be provided with some sample recipes for preparation. Those who find it unacceptable will be excluded from the study.

On enrollment in the study, quantitative dietary intake of children will be assessed using a multiple-pass, 24-hour intake on two separate days. Characteristics of interest will be percent of calories as protein, fat, and carbohydrates, intake of saturated, unsaturated, and trans fatty acids, ratio between total omega-6 and omega-3 fatty acids, and intake of antioxidant nutrients. Usual medications and dosages will be listed and participants will be asked to keep a record of any as-needed asthma medications that they use during the study. Asthma severity will be assessed using pulmonary function tests (part of routine clinical care) and a symptom severity score. Clinic personnel will be blinded as to treatment group. Body mass index (BMI) will be calculated from height and weight measured in clinic. BMI may be a factor in airway reactivity so it will be included as a variable in the data analysis.

Children randomized to the high omega-3 group will be provided with individually frozen trout fillets and instructions to prepare the trout any way other than frying it. Their

families will be taught other ways to increase the intake of omega-3 fatty acids, such as substituting canola oil for soy or corn oil. Both groups will continue to receive their usual care in the clinic. The intervention will continue for 12 weeks. Longer than that may become burdensome for the families and in an EPA supplementation study, six weeks was enough time to saturate cell membranes with EPA (Simopoulos, 2002). Acceptance of the fish and compliance with study protocol will be assessed by family report at 4, 8, and 12 weeks. Assessment of diet, medication use, and asthma severity will be repeated at the end of the study. The two groups will be compared for dietary intake, ratio of omega-6 to omega-3 fatty acids, episodes of asthma symptoms, changes in medication usage, and changes in pulmonary function tests and symptom severity scores.

Personnel:

Cindy Fitch – WVU, Human Nutrition and Foods Faculty
Ken Semmens – WVU, Extension Faculty
Megan Moran – Graduate student in HN&F program

Institutional Units Involved:

WVU Animal and Nutritional Sciences
WVU Extension
WVU School of Medicine

Objective 6: Effect of feed containing *Phaffia rhodoxyma* on the external appearance and flesh color of rainbow trout.

Justification

Colorful trout appeal to both the angler and the consumer, but synthetic pigments carry a stigma in today's market. Salmon diets are supplemented with synthetic Astaxanthin or Canthaxanthin to impart a red color to the fillets. Given the high cost of synthetic pigments, and the desire of consumers for natural ingredients, there is demand for other ways to add color to salmonids. Igene Biotechnology has developed a natural product based on a culture of a red yeast, *Phaffia rhodoxyma*. Learning how to mill and apply this material may benefit both the food and recreational trout market in the region. It is expected that lower temperature of the vacuum coating technique will result in less pigment loss as the product is incorporated into the feed and greater predictability in pigment content than is the case with extruded products. This study proposes to apply natural pigment in a controlled manner to grow out of rainbow trout. Improving the external appearance of the fish is expected to influence marketability of trout in the recreational market. Improving the color of the flesh with natural pigments is expected to provide another tool for niche marketing locally grown trout.

Previous Work and Present Outlook

Astaxanthin is not commonly used in trout feeds, but it is known to work well. Native brook trout in streams where crustaceans are an abundant food source are known for having flesh with a reddish pigment. Though adding pigments to the feed increases the cost of the fish, coloration of the fish and the meat may provide a market advantage in both the food and recreational market that may offset the higher production costs.

Current work with brook trout fed flax oil supplemented feed supports niche market development and the strategy of adding value.

Objectives

1. Measure the growth, survival, feed conversion of rainbow trout fed each of the diets.
2. Document the external color of golden and normal rainbow trout in each treatment at 6, 12, and 18 weeks.
3. Compare the fillet color of rainbow trout fed these diets for 6, 12, and 18 weeks.
4. Improve capacity for the Aquaculture facility at Reymann Memorial Farm to demonstrate production technology and methods.

Procedure

This experiment will use approximately 6000 fingerling rainbow trout (normal and golden) approximately eight inches total length. Six raceway tanks supplied with aerated spring water will be partitioned to create 12 experimental units of equal size. There will be 4 treatments (Control, 20, 40, and 60 ppm Astaxanthin) with three replications per treatment.

Igene Biotechnology will engage Zeigler Bros, Inc, to produce the experimental diets. A vacuum coater will apply pigment into a commercial trout feed. Samples of each diet will be provided to Igene at six week intervals. Igene will analyze the feed for Astaxanthin content in their laboratory.

The production experiment will be conducted at the Reymann Memorial Farm aquaculture facility. Fingerling rainbow and golden trout will be obtained from commercial producers, acclimated to the system, and segregated into 12 equal groups. Fish will be fed daily using a feeding schedule adjusted on a daily basis - the same schedule used by the RDSS program. As such this trial will provide information to verify the RDSS program. Feed will be weighed out daily for each tank. Amount fed, oxygen concentration in each tank, and mortalities will be recorded on a daily basis. Data regarding growth rate, survival, length frequency, condition factor and feed conversion will be collected at six week intervals during the culture period. At this time, Six fish from each treatment will be taken to monitor pigmentation of the flesh will also be taken. Half of the fish will be sent to Igene Biotechnology for astaxanthin analysis, and half will be transported to WVU campus in Morgantown where they will be filleted and photographed.

Documenting the external color of live fish will be done with photography. A professional photographer with West Virginia University will travel to the Reymann Memorial Farm at 6, 12, and 18 weeks. Photographs of live fish from each treatment will be taken. The experiment will be terminated at 18 weeks, as the fish reach a total weight of 1 lb. Fish will be made available to other investigators to use if there is an interest.

Facility Improvements There is value in applying technologies developed for recirculating systems to the flow through system at Reymann Memorial Farm. Circular tanks have always had the advantage of removing solid waste quickly. Drains have been

developed for the center of the tank that separate the outflow into two streams. One stream is low volume and concentrates the solid waste, and the other stream is high volume and has a low concentration of solid waste. This technology will integrate well with the system currently in place. The concentrated waste would be diverted to the sludge sump and thence to the geotube. The circular tanks would be placed in the building across from the raceway such that a vehicle could drive between the tanks down the center of the building. By pumping water from the pond through the tanks, we would be demonstrating ways to reuse the water in a hybrid system. Increasing the number of tanks also provides more flexibility for training, conducting experiments, holding different groups of fish, and perhaps growing fingerlings.

Financial Support

Igene Biotechnology will donate the feed used in this study and conduct pigment analysis of the feed and fish flesh.

Personnel

Ken Semmens – WVU Extension Faculty, Animal and Nutritional Sciences
James Montgomery – WVU, Photojournalist
Pat Monahan – Igene Biotechnology
Chestina Merriner – Reymann Memorial Farm
Rodney Kiser - WVU Extension.

Institutional Units Involved

WVU Extension Service
Division of Animal and Nutritional Sciences
Reymann Memorial Farm
WVU Photographic Services

Objective 6. Aquaculture Extension

Justification

Production of aquatic plants and animals is feasible by the homeowner and the commercial producer. The diverse products produced by aquatic culturists include food, bait, sport fish, ornamental fish, plants, and even fish which control aquatic weeds. Aquaculture is a new concept to many residents of West Virginia and it is growing slowly. The industry is emerging as a sustainable and potentially profitable way to produce agricultural crops commonly considered wildlife. West Virginia has water resources and topography well suited for small scale production of trout. Aquaculture extension at WVU is closely integrated with the Aquaculture Product and Marketing Development Project (APDMP). This USDA/CSREES funded project originally focused on mine water and recreational markets. Subsequently the focus was broadened to flowing water systems and niche markets.

The need for extension activities associated with APDMP remains strong. Results from a wide series of projects must be presented to existing and prospective producers, and the state agencies influencing aquaculture development. Presentations are also made to a

diversity of groups at state and national meetings. Requests for literature and information have doubled since the inception of the project. There is greater demand for site visits to assess opportunities, and assist individuals growing or utilizing farm raised fish.

Previous Work and Present Outlook

Capacity for WVU to serve the aquaculture industry has increased significantly. Faculty and staff aquaculture expertise has increased significantly. Two pilot scale raceway facilities have been developed. The system known as Dogwood Lake is about 15 miles west of the WVU campus on property owned by Consol Energy, Inc. and uses treated mine water to produce trout on a seasonal basis. A second system near Wardensville, WV utilizes spring water and is located at the Reymann Memorial Farm, part of the WV Agricultural and Forestry Experiment Station.

West Virginia leads the nation in utilizing groundwater from coal mines for aquaculture production. Mine water is the primary water source for production of farm raised food fish in the state. The project has collaborated with several energy companies (Consol Energy, Eastern Associated Coal Co., Duquesne Power & Light, Martinka Coal Co.) to develop acid mine drainage sites for aquaculture production. These companies have realized savings in reclamation costs as they accommodate this new form of agriculture. Research has shown how rainbow trout benefit from the mineral content of water from an Acid Mine Discharge treatment plant. This explains why it is possible to use treated mine water to grow trout when one might expect the fish to die. In Monongalia County, approximately 8,000 lb of trout have been grown in the demonstration system each winter. Fish grown in the system were tested for contaminants and were shown to be safe to eat. For the first time in West Virginia and perhaps nationally, aquaculture was written into the reclamation plan for a coal mining operation in Boone County. WVU aquaculture personnel collaborated in the design and installation of a small raceway system suitable for trout production at the site. In Marion County, a retired acid mine drainage treatment plant has been shown to be suitable for use as a community fishing park. In 2006, the Marion County Commission acquired the site for development as a fishing park.

Continued funding of APMDP has allowed time for leadership to impact aquaculture infrastructure in West Virginia. Staff with the State Veterinarian's office collaborate with WVU aquaculture investigators to develop capacity to test for fish pathogens and develop a system to certify farms free from specific pathogens. We continue to work with Senator Walt Helmick and various state agencies on a proposal to purchase 250,000 pounds of trout annually from WV fish farmers and stock them into public waters to enhance tourism. We collaborate with fish farmers to support legislative changes that will make waste management less costly and cumbersome to the producer. Investigators reach extends far beyond the confines of WVU campus. Municipal staff in Franklin, Bridgeport, and Ripley are provided guidance to organize fishing events. Working with a local producer and his church family, we will continue to facilitate development of healthful trout products and serve them to the public at venues like the Jefferson County Fair.

Objectives

1. Conduct educational programs, develop information and provide assistance to the aquaculture industry in three basic areas:
 - Production.
 - Marketing.
 - Infrastructure development
2. Operate and improve flowing water systems at Dogwood Lake and the Reymann Memorial Farm.

Procedures

Many different methods are used to reach a diverse group of stakeholders. The year begins with a state wide meeting known as the Aquaculture Forum. Throughout the year, fish are grown at two locations with flowing water systems. These facilities are utilized for research and demonstration and are made available to classes, visitors, and for workshops. An aquaculture class is taught to WVU graduate and undergraduate students. This summer an online course on Recreational Pond Management will be offered. A web site is maintained for extension and for communicating research results. Software is developed to assist with making complex management decisions. A quarterly newsletter is distributed and articles are submitted to popular publications. Responses to requests for literature and information are made via email, and telephone. When these methods are not sufficient, site visits are scheduled to meet directly with the stakeholder. Research results are presented at regional and national meetings and published in scientific journals.

The program directly serves current and future West Virginia producers of fish for food and recreational purposes and processors of food fish and has conducted activities in more than 15 counties. Over 100 individuals annually attend the Aquaculture Forum and other aquaculture presentations and approximately 400 requests for information are received annually. Over 100 visits to specific sites are conducted annually. Aquaculture extension occasionally services such as water quality testing, testing for contaminants, and disease diagnosis.

Development of the Mine Water Resource. Mine water has emerged as the most important source of water for commercial production of salmonids in West Virginia. Each site must be evaluated on its unique topography, water quality, water volume, etc. We continue to engage two large coal companies and numerous mine sites. We will continue to work with coal companies and economic development agencies the step by step process of site assessment and education required for development of the mine water resource. We will coordinate entrepreneurs to assist with determination of production capacity and appropriate facility design once a site has been chosen for development.

The individual responsible for this area of work will coordinate with various investigators to develop workshops, a newsletter, and publications useful for development of the mine water resource. He will also be responsible for management of the demonstration raceway at dogwood lake and installation of a geotextile bag to recover solid waste.

Aquaculture and Recreation. Most fish farmers in West Virginia sell their fish in the recreational market. Approximately 300,000 lb is sold to fee fishing businesses around the state. In addition, live fish are sold to fishing clubs, large companies, housing associations, and private individuals for recreational use in both private and public waters. Specific efforts have recently developed creating an increased demand for work in this area. We are collaborating with Senator Walt Helmick regarding a fish stocking initiative to enhance tourism and continue to collaborate with other investigators for developing fishing packages at Pipestem Resort. Building upon the data developed in previous research, it is timely that this information be combined with the experiences of fish farmers serving the recreational industry and extend it to a wide variety of recreational stakeholders. Such stakeholders would include resorts, attractions, fishing clubs, communities developing strategies to enhance tourism, and the general public. Site visits and assistance to farmers targeting the recreational market will also be an important component of this work. There is opportunity to develop partnerships between segments of the tourism industry, fish farmers, fisheries managers, and the resource base to respond to the recreational opportunities described by marketing research. The aquaculture facility at Reymann Memorial Farm will increasingly be utilized as an educational venue for production of fish in the recreational market.

Aquaculture Extension personnel will disseminate information generated by this project to the aquaculture industry in Appalachia, to government agencies with aquaculture-related responsibilities, and to the general public. Investigators will continue to collaborate with the Freshwater Institute's Aquaculture Program, the West Virginia Department of Agriculture, the West Virginia Aquaculture Association, and other organizations to deliver research findings in the most user friendly manner possible.

Personnel

This project is led by Ken Semmens, the Extension Specialist for Aquaculture. The project provides salary for Rodney Kiser, a Research Assistant who focuses on trout and recreational use of farm raised fish, and Dan Miller, a Research Associate in the Division of Resource Management who focuses on developing the mine water resource for aquaculture. Occasionally other state extension specialists contribute to aquaculture programming, particularly the weed specialist addressing aquatic weed management. County agents typically report about 60 days in support of pond management and aquaculture annually.

Institutional Units Involved

Primary Funding for WVU Aquaculture Research and Extension is through a USDA/CSREES grant with the Davis College of Agriculture Forestry and Consumer Sciences (DCAFCS). The following faculty will participate in this project:

- Marketing – Cy Logar
- Animal and Nutritional Sciences
 - Animal Nutrition – Ken Blemings
 - Human Nutrition - Kristen Matak
 - Protein Recovery – Jacek Jaczynski.
 - Production – Ken Semmens

- Resource Economics
 - Economics - Gerard D'Souza, Cheryl Brown
 - Review WV legal structure - Denny Smith, Fonda Holehouse
- Engineering
 - Environmental – Donald Gray
 - Chemical – Richard Turton
- Recreation and Parks – Chad Pierskalla
- Horticulture – Todd West

Cooperation.

Collaboration has developed with a variety of groups listed below.

- Producers
- Mining Companies
- NGO's
- Economic Development Authorities
- State Agencies
- Aquaculture Suppliers
- Processors
- Higher Education

Project Timetable

The work described in this proposal will be completed by the end of September, 2008. Most components will begin in the summer of 2007 and be completed a year later. The legal research and work will span a period of 15 months.

Financial Support

This project will be coordinated with the APMDP to continue the current program. The budget for this proposal is summarized below.

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