

IV. PROGRESS REPORTS

**A. FOOD SAFETY AND SANITATION
OF AQUACULTURAL
PRODUCTS: MICROBIAL**

Progress Report
For the Period
April 1, 1992 to August 31, 1995

FUNDING LEVEL:

Year 1	\$ 85,000
Year 2	\$225,000
Year 3	\$260,000
Total	\$570,000

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PROJECT OBJECTIVES:

1.a. Collect data that are available to define
aquacultured food safety problems and to design
a control program.

b. Conduct a forum to assess all relevant
data on food safety of aquacultured foods. This
event will assemble all knowledgeable individuals
that can bring their expertise to bear on this
subject.

c. Prepare and distribute a bibliography of
the available publications, mimeographs, fact
sheets, and videos relative to food safety and
sanitation in the aquaculture industry.

d. Evaluate data on microbiological quality
in catfish, crawfish, and rainbow trout processing
and distribution operations. Determine if there
are critical control points which need attention.

e. Do supplemental laboratory work to
clarify areas of concern. This is designed to fill
gaps in the database, not to conduct an industry-
wide survey.

2. Investigate various methods to reduce
and detect significant pathogenic and spoilage
micro-organisms on processed catfish, rainbow
trout, and crawfish. Coordinate findings with
publications work group if necessary.

3. Conduct a food safety HACCP audit to
determine if this approach would be cost-effective
and result in increased product safety.

4. Produce new publications to complement

existing publications on food safety and sanitation. These would be completed during the second and third years of the project.

OBJECTIVE 1A: COLLECT DATA THAT ARE AVAILABLE TO DEFINE AQUACULTURED FOOD SAFETY PROBLEMS AND TO DESIGN A CONTROL PROGRAM.

PROGRESS:

Efforts to assess the food safety of southern aquacultured products based on reported illnesses, literature reviews, and liaison with numerous related government programs still reveal that cultured fish represent the safest source of muscle protein and related nutrients among all muscle foods produced in the United States. Cultured molluscan shellfish, which in the southern region are primarily hard clams, are more suspect for potential microbial foodborne illnesses, yet actual reported illnesses do not reflect any significant reporting of occurrences from cultured molluscan products. Likewise, shrimp and crawfish, as cooked ready-to-eat items, are suspect, but not evidenced as problems. These conclusions are based on updated literature reviews per the previously identified sources through 1995 and supplements from pertinent agencies in regional State and Federal programs.

The few reported illnesses associated with consumption of clams, *Mercenaria campechensis*, harvested in Florida involved encounters with the potential pathogen bacteria, *Vibrio vulnificus*. These limited illnesses typically involve 'at-risk' consumers in that they have health conditions that compromise their immunity to infections introduced by consumption. These pathogens are indigenous to southern coastal waters approved for shellfish production, particularly during warmer months (April - October). In order to help reduce the levels of *Vibrio vulnificus* on oysters and related shellfish, the Interstate Shellfish Sanitation Conference (ISSC) introduced (August 1995) a new time-temperature harvesting scheme to retard growth of the bacteria on harvested products. The new harvesting

guidelines, linked to growing water temperatures, are referenced in the Total Quality Assurance (TQA) and Hazard Analysis and Critical Control Point Manuals generated, in part, by this SRAC project (Otwell and Garrido, 1995).

Agency liaison through this project has matured into a number of collaborative projects to implement respective control measures for aquatic food product safety during processing. Meetings with the pertinent regional State agencies and their professional association, AFDOSS (Association of Food & Drug Officials of Southern States), have led to a formal partnership called the "Seafood HACCP Alliance for Education and Training". This partnership, formalized in June 1994 with support from the National Sea Grant Office, has developed a network of Cooperative Extension Services and related Sea Grant Advisory Programs working with representatives from the FDA Office of Seafoods, USDA, National Marine Fisheries Service, and all respective regional AFDO (Association of Food & Drug Officials) affiliates to design and deliver a uniform HACCP education and training program for all aquatic food processors. Aquatic foods include all seafoods (harvested, cultured or imported).

SRAC's Objective 1a provided the initial opportunity for the project personnel to draft and advance the "Alliance" concept in conjunction with the AFDOSS (AFDO of Southern States) organization. This educational "Alliance" will offer continuing controls for aquacultured product safety through the joint development of 'core HACCP curriculum', establishment of a cadre of HACCP instructors, pilot-testing in processing firms, and maintenance of a 'compendium' of approved processing methods and recommendations for HACCP monitoring and record keeping. To date the investigators have assisted in preparation of HACCP training modules for processing of molluscan shellfish and crawfish, which can include cultured products. This involvement assures a role for SRAC in providing essential HACCP training for aquacultured production and processing in southern states.

Concurrent with the aforesaid activity, this project has helped foster regional collaboration in a joint USDA Extension Service project, "Implementation of TQA and HACCP Concepts for Processing Aquacultured Products". This project has been completed with the development and in-plant testing of HACCP programs for cultured molluscan shellfish (University of Florida - Steve Otwell with Louisiana State University - Mike Moody) and catfish (Mississippi State University - Anna Hood with Virginia Tech - George Flick). The second year of work will continue with cultured crawfish (LSU - Mike Moody with University of Florida - Steve Otwell) and trout (Virginia Tech - George Flick with MSU - Anna Hood). The SRAC project initiated this collaboration. Likewise, as a consequence of this work the SRAC investigator has been asked to serve as the Chairman of the Interstate Shellfish Sanitation Conference's HACCP Committee to investigate the integration of proposed HACCP concepts and regulations within the existing Federal manuals which govern the production and processing of all cultured and natural harvested bivalves.

Two HACCP manuals (Otwell and Garrido, 1995) for cultured shellfish have been generated by this work in conjunction with SRAC.

WORK PLANNED:

All project work has been completed. A final "Train-In-Place (T.I.P.)" training program to assist TQA & HACCP implementation in aquaculture production and processing will be included in the SRAC project termination report.

IMPACTS:

Reviews of previous and current literature and data sources further substantiate the food safety status for southern aquacultured products. Project activity helped found a national "Seafood HACCP Alliance for Education and Training" which will lead to more uniform implementation of control measures for aquacultured product safety. Project activity also fostered cooperative

projects funded by USDA to implement HACCP programs in actual aquaculture process settings for cultured bivalves, catfish, crawfish and trout.

Manuals for commercial guidance in implementation of TQA & HACCP programs are now available for cultured oysters and clams.

OBJECTIVE 1B: CONDUCT A FORUM TO ASSESS ALL RELEVANT DATA ON FOOD SAFETY OF AQUACULTURED FOODS. THIS EVENT WILL ASSEMBLE ALL KNOWLEDGEABLE INDIVIDUALS THAT CAN BRING THEIR EXPERTISE TO BEAR ON THIS SUBJECT.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

An *Aquaculture Safety Forum* was held February 2-4, 1993, at the Auburn University Hotel and Conference Center. The two-and-a-half-day *Forum* brought approximately 45 industry, academic, and government agency representatives from 11 southeastern states together to assess all the relevant data available on the safety of aquacultured foods. The plenary sessions provided opportunities for aquaculture researchers and Extension workers to update the group on recent research findings and other current topics. The breakout sessions afforded an opportunity for *Forum* participants to develop "White Papers" about the present status and future needs of the chemical and microbial aspects of aquacultured foods in the southeastern United States. All *Forum* participants received an evaluation form on which their perceptions of various aspects of the *Forum* could be rated on a scale of 1 (=Poor) to 5 (=Very Good). Forty percent of the evaluations were completed and returned. The following ratings are presented as means +/- standard deviations: the overall format of the *Forum* received a very favorable rating of 4.72 +/- 0.45. The attendees were also favorably impressed with the strength of the agenda (4.67 +/- 0.47) and the quality of the speakers (4.61 +/- 0.49). Evaluation respondents indicated that the degree to which the *Forum* addressed the

issue of aquaculture products safety merited a rating of 4.56 +/- 0.60.

A 157-page *Proceedings of the Aquaculture Products Safety Forum* was produced. The *Proceedings* included transcripts of 20 formal presentations made during the plenary sessions, plus recommendations made by working groups regarding microbial and chemical safety of aquacultured food products. Nearly 275 copies of the *Forum Proceedings* were distributed to extension workers, researchers, and government agency representatives in 33 states plus Puerto Rico and the Virgin Islands. While no formalized method was established to evaluate the *Proceedings*, informal comments were positive with regard to content, utility, layout, and design of the *Proceedings*.

A 60-minute live, interactive *Aquaculture Products Safety* satellite videoconference was produced which highlighted the objectives and recommendations developed during the *Forum*. Among the issues discussed were: Hazard Analysis of Critical Control Points (HACCP) method of fishery product inspection, microbial aspects of aquaculture safety, and chemical residues and their relation to aquaculture safety. Another portion of the videoconference presented videotaped excerpts of interviews (conducted during the forum) in which the interviewees discussed what they perceived as the greatest needs related to aquaculture safety, and what would be the most appropriate ways to address those needs. A final segment of the program was devoted to questions and answers, some of them phoned in from interested viewers.

Videoconference Evaluation Forms were sent to each Alabama Cooperative Extension Service County Office. Although relatively few of the forms were returned, those who did respond felt the videoconference was worthwhile. Ratings of the panelists' presentations ranged from "useful" to "very useful". The interactive segment of the program was deemed beneficial, with viewers feeling "somewhat involved". The technical

quality of the production received ratings that ranged from good to excellent.

IMPACTS:

Participant and viewer evaluation results were mentioned previously and demonstrate the very positive impacts that Objective 1b had on three distinct audiences. Since the conclusion of the "Forum Project", many researchers, Extension workers, and government agency personnel have commented as to the "focusing" effect produced by the *Forum*. Many believe that the *Aquaculture Products Safety Forum* helped to reduce the amount of overlap and increase the complementary nature of subsequent aquacultured products research and Extension efforts in the Southeast.

OBJECTIVE 1C: PREPARE AND DISTRIBUTE A BIBLIOGRAPHY OF THE AVAILABLE PUBLICATIONS, MIMEOGRAPHS, FACT SHEETS, AND VIDEOS RELATIVE TO FOOD SAFETY AND SANITATION IN THE AQUACULTURE INDUSTRY.

ANTICIPATED BENEFITS:

The bibliography on available information on food safety and sanitation as related to the freshwater aquaculture industry will provide a vital resource to those interested in these topics. The information will be available both in the printed form and on computer discs.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Academic institutions, governmental agencies and companies involved in processing aquacultured products were contacted. Requests were made to notify the researchers of any documentation within their groups that pertained to food safety and sanitation as related to the aquaculture industry. Information to be included in the bibliography has been compiled and final corrections are being made. Copies of the bibliography will be made and distributed to those involved in freshwater aquaculture.

WORK PLANNED:

Objective completed.

OBJECTIVE 1D: EVALUATE DATA ON MICROBIOLOGICAL QUALITY IN CATFISH, CRAWFISH, AND RAINBOW TROUT PROCESSING AND DISTRIBUTION OPERATIONS. DETERMINE IF THERE ARE CRITICAL CONTROL POINTS WHICH NEED ATTENTION.

ANTICIPATED BENEFITS:

The aquaculture industry will benefit directly from technical information generated in this project. Methods were developed and tested to identify control points during processing that result in increased microbial loads and to design alternatives to the process to enhance the overall microbial quality of processed aquaculture products. Methods were also developed to inhibit the proliferation of the human pathogen *Listeria monocytogenes* on ready-to-eat crawfish tail meat, including modified atmosphere packaging, heat pasteurization, and a combination of physical and chemical treatments. Vacuum skin packaging, an advanced packaging system, was shown to improve shelf life and product appearance of rainbow trout by inhibiting the production of *Clostridium botulinum* type E toxin. These results will help the industry in distribution and retailing and in developing standard procedures and methods for the examination of processed products to monitor and maintain microbial quality and safety.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Evaluation of microbial data (aerobic plate counts, total coliforms, and *E. coli*) representing 5 replicate samples from each stage of processing collected on 5 separate occasions over a one and one half year period has revealed a critical control point. The magnitude of the increase observed at the critical control point in processing is not significantly different between seasons. Process modifications evaluated to date have not

significantly reduced the impact of the critical control point on the overall microbial load. Additional process modifications, such as chlorine sprays, are currently being evaluated by the processor as a means of reducing microbial loads after the critical point in processing.

Sampling of processing plants during different seasons demonstrated that aerobic plate, total coliform and *E. coli* counts are affected by season. During warmer months of spring and summer, all parameters measured increased 10- to 100-fold. The increase was associated with higher surface microbial loads of catfish entering the processing plant. Although pond water was not analyzed, a change in water quality during the warmer months is probably the origin of the increase, because the level of microorganisms on fish is usually a function of the microbial content of the water. Methods of controlling waste production, excess nutrients and other parameters within the catfish ponds during this critical time period should be evaluated to determine if initial surface microbial loads of catfish can be reduced prior to entering the processing plant.

A pathogen survey of fully processed catfish fillets was conducted in conjunction with Virginia Polytechnic Institute and State University and Mississippi State University. Twenty fully processed fillets from each of 3 processing plants were collected on a quarterly basis for one year. Investigators from Auburn University were assigned analyses for the following three pathogens: *Edwardsiella*, *Salmonella*, and *Shigella*. *Edwardsiella*, which is primarily a pathogen of fish, was isolated from 12.3% of 220 fillets sampled. *Salmonella* and *Shigella* were less common and were isolated from 2.3% and 1.8% of fillets sampled, respectively. A slight seasonal increase in the number of fillets harboring *Edwardsiella* was observed, with 63% of the positives occurring in the spring and summer. A similar trend was not observed for *Salmonella* and *Shigella*, which were isolated on rare occasions throughout the year. The incidence of *Salmonella* and *Shigella* on catfish is lower than reported incidences for most other raw meats.

Glycerol monolaurate (monolaurin) inhibition of *L. monocytogenes* was affected by pH and testing medium; monolaurin activity increased as pH decreased. Monolaurin interacted additively with citric acid and synergistically with acetic acid, benzoic acid and lactic acid to inhibit *L. monocytogenes*. For growth prevention or destruction of *L. monocytogenes* in crawfish tail meat, 224 or 336 mM lactic acid was required, respectively. Destruction of the bacterium could be achieved with 224 mM lactic acid when 0.72 mM monolaurin was added.

Citric acid or potassium sorbate sprays applied to crawfish tail meat to a final concentration of 0.03 g/kg did not prevent growth of *L. monocytogenes* at 4°C. Potassium sorbate did, however, extend lag phase of the bacterium by 2 days. Thus, these treatments were not effective to control the bacterium.

Modified atmosphere packaging (MAP; 74.8% CO₂, 10.4% O₂, and 14.8% N₂) inhibited growth of *L. monocytogenes* in crawfish tail meat treated with 0 and 1% lactic acid (LA) and stored at 4°C when compared to air and vacuum packaging. No differences in effectiveness of the packaging atmospheres were observed with 2% LA. Addition of 200 µg/g glycerol monolaurate (ML) with 1% LA inactivated *L. monocytogenes* for 20 d at 4°C in each packaging atmosphere. This treatment reduced pH from 7.4 to 5.4.

Lactic acid addition to crawfish tail meat could increase the resistance of *L. monocytogenes* pasteurization temperatures. D₆₀ values of *L. monocytogenes* in tail meat treated with 0, 0.5, 0.75, and 1% lactic acid were 4.68, 4.41, 3.46, and 2.49 minutes, respectively. Atmosphere surrounding the tail meat, whether air, O₂, CO₂, or N₂, had no apparent effect on heat resistance of the bacterium. *L. monocytogenes*, at levels naturally occurring, can be eliminated from crawfish tail meat by treatment with heat alone (60°C for 15 minutes) or heat (60°C for 8 minutes) combined with 1% lactic acid. Crawfish tail meat dipped in 1% lactic acid were brighter in color

(enhanced redness and whiteness), had reduced fishy odor, and were firmer than untreated controls. These character changes were not disliked by taste panelists.

The effects of monolaurin and lactic acid, singly or combined, on *L. monocytogenes* attached to catfish fillets revealed that monolaurin up to 400 µg/ml had no influence on counts. Conversely, lactic acid-treated fillets had reduced counts compared to controls. Dipping in 0.85, 1.70, or 2.55% lactic acid for 30 minutes reduced counts by 0.9, 1.4, or 1.3 logs, respectively. Extending dipping time to 60 minutes resulted in little additional decrease in counts. Combining monolaurin with lactic acid yielded results similar to lactic acid alone. Hence, population reduction ability of the two compounds resides with lactic acid and not monolaurin.

Planktonic and adherent cells of *L. monocytogenes* were subjected to heat, monolaurin and acetic acid to evaluate biofilm removal from stainless steel. Planktonic cells were more sensitive than attached cells to the physical and chemical treatments. Effectiveness of 100 µg/ml monolaurin on destruction of biofilm cells was increased when combined with heat (60°C) or acetic acid (1%). Old biofilm cells (7 days) were more resistant than young biofilm cells (1 day) to the treatments. Cells in a rich nutrient environment were more resistant than those in a depleted nutrient environment. Results suggest that eradication of cells in biofilms is more easily accomplished when biofilms are young. Processors of aquacultured products should have frequent, routine cleaning and sanitation programs to minimize biofilm problems.

The growth and toxin production of *C. botulinum* type E in rainbow trout fillets held in vacuum skin packaging indicated no toxin was produced by *C. botulinum* type E in fillets stored at <3°C. This advanced packaging method improved the shelf life and product appearance. The use of modified atmospheres at 10°C had little practical usefulness. Carbonic acid dips

caused a slight reduction in microflora of trout (approximately a ½ log reduction); however, the effect was negated by the additional handling and cost involved in preparation of the dip. No *Salmonella* or *Listeria* were detected in any sample of rainbow trout during the study using FDA and USDA isolation and confirmation protocols.

Higher concentrations of CO₂ (60% and 100%) in modified atmospheres with no addition of O₂ extended shelf life of trout fillets at least 7 days longer than trout packaged in atmospheres containing O₂ at 3°C. The presence of O₂ in atmospheres encouraged growth of aerobic bacteria, psychrotrophic bacteria, yeasts, aerobic sporeformers, coliforms, proteolytic and lipolytic bacteria. The odor and appearance of fillets packaged in atmospheres containing O₂ were significantly less acceptable (p<0.05). Proteolytic and lipolytic bacteria were extremely sensitive to high CO₂ atmospheres resulting in a 4 to 6 log difference after 10-15 days of storage. The more rapid spoilage in trout packaged in O₂ containing atmospheres was probably due to breakdown of amino acids, fatty acid and non protein nitrogenous compounds by lipolytic and proteolytic bacteria.

WORK PLANNED:

Investigations are in progress to evaluate the use of edible films to improve microbial quality of smoked rainbow trout. Growth and toxin production of *C. botulinum* type E on modified atmosphere packaged rainbow trout and catfish are being studied; the application of time and temperature indicators on packaged aquaculture products is being evaluated. The investigators will continue to communicate data and ideas to producers and processors to further improve the quality and safety of catfish from the farm to the consumer.

IMPACTS:

Processing steps have been identified where

microbial counts increase faster than at other processing sites, which makes these sites targets to control microbial proliferation, thereby improving quality and microbial safety of the processed product. The project has focused directly on conditions which promote quality and safety of marketed aquaculture products. The training of processing plant personnel during the collection of base line data and the feedback of data to the industry will have a major impact on successful implementation of the HACCP concept and on the safety of aquaculture products.

Methods have been tested for the prevention of growth or destruction of the human pathogen *L. monocytogenes* on precooked ready-to-eat crawfish tail meat and the inhibition of *C. botulinum* toxin in rainbow trout fillets. These methods can prevent costly foodborne outbreaks associated with these bacteria.

It has been demonstrated that the shelf life of rainbow trout can be extended by one week at 3°C by packaging with 60% CO₂ and 40% N₂. Also, packaging of trout under 60% CO₂/40% N₂ did not significantly increase anaerobic spore counts during the 21-day storage period at 3°C.

OBJECTIVE 1E: DO SUPPLEMENTAL LABORATORY WORK TO CLARIFY AREAS OF CONCERN. THIS IS DESIGNED TO FILL GAPS IN THE DATABASE, NOT TO CONDUCT AN INDUSTRY-WIDE SURVEY.

ANTICIPATED BENEFITS:

The project will provide the aquaculture industry with the necessary database and expertise to improve the microbial quality and safety of aquaculture products and to aid in the implementation of industry obtainable standards and effective HACCP programs.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Data collected from each stage of processing from whole fish to finished product reveal that

the overall microbial load on catfish increases by 100- to 1000-fold during processing. Three conditions are chiefly responsible for the increase: (1) contamination of flesh from equipment surfaces after the skin is removed, (2) growth of contaminating organisms during processing and holding, and (3) cross contamination of fillets during specific stages of processing. Analysis of farm raised catfish fillets from several retail markets indicated an additional 100-fold increase in the microbial load during distribution and final marketing. Although differences in aerobic, total coliform, and *E. coli* counts were evident among retail markets, it is not possible from these data to determine the cause of the higher microbial loads. The incidence of three pathogens, *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* species on retail catfish fillets was also determined. *L. monocytogenes* was isolated from 5 fillets (5.4%), no *E. coli* O157:H7 was detected and a *Salmonella* species was isolated from one fillet (1.1%). Contrary to expected trends, no correlation existed between retail markets with high aerobic, total coliform and *E. coli* counts and the incidence of the three pathogens. Data collected from these studies continues to build a database essential in determining the stages of processing, distribution and marketing which significantly impact the microbial quality of farm raised catfish and can be used to develop alternative processing and handling procedures to further improve the microbial quality and safety of catfish products.

IMPACTS:

Substantial resources in terms of expertise and manpower have been provided to build a microbial database for processed catfish, which were previously not available to the aquaculture industry.

OBJECTIVE 2. INVESTIGATE VARIOUS METHODS TO REDUCE AND DETECT SIGNIFICANT PATHOGENIC AND SPOILAGE MICRO-ORGANISMS ON PROCESSED CATFISH, RAINBOW TROUT, AND CRAWFISH. COORDINATE FINDINGS WITH

THE EDUCATIONAL PUBLICATIONS WORK GROUP IF NECESSARY.

ANTICIPATED BENEFITS:

Catfish processors have continued to express an interest to initiate a total quality assurance program to meet present and future market demands for increased quality assurance of fresh and processed products. To assist the processors in this effort, a microbiological based quality evaluation was performed on finished products. The microbiological based quality evaluation program included a routine microbial evaluation of the processed products for selective indicative bacteria (aerobic, fecal coliform and *E. coli* counts). Currently there are no standards in the United States for dressed fresh/frozen catfish products using indicative microbes as the criterion. However, there are general standards for fresh fish products developed by The International Commission on Microbiological Specifications for Foods. Wholesale distributors in the United States and Canada are stipulating purchase specifications for catfish products that processors must meet in order to prevent their rejection. These specifications are particularly directed toward *E. coli* counts even though all *E. coli* are not pathogenic. Unfortunately, the buyer's specifications that reflect high standards may not be consistently achieved during processing. The indicative microbiological quality control program could define the development of realistic and achievable standards. Processors could use these standards to market their products while assuring both product quality and safety.

To illustrate the benefit, if a buyer specifies a product count less than x cfu/g and the cfu/g are lower than the specifications by 10 fold, the processor may be able to request a premium price for the higher quality. Domestic consumers, however, are less quality conscious than the Canadian, European, and Japanese consumers. The European and Japanese consumers will pay premium prices for product quality. Processors have expressed a desire to expand their markets

to Europe and Japan where, generally, the standards are higher. Also, these markets are desirable since they have the potential for greater profits. Thus, an indicative microbiological quality control program could be beneficial for the processor.

Listeria monocytogenes contamination of foods remains the focus of vigorous efforts by government and industry concerned with food safety, especially that related to ready-to-eat products. In a highly competitive market, as in the catfish industry, processes that could reduce initial numbers of bacteria on fillets or extend shelf life and improve product safety would result in a more stable economic environment for the catfish processor. Due to the decline of red meat consumption and steady increase in aquaculture fish consumption, this information may be vital to the industry by providing direction for future quality control programs.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Both indicative and pathogenic microbial flora of fresh aquacultured channel catfish fillets were sampled to evaluate the microbiological quality. Three catfish processors participated and were selected with consent to represent small through large processors. Products were screened during four different seasons (e.g., summer, fall, winter and spring); there were significant differences in the microbiological quality of fillets due to processing conditions and to production seasons. All of the processors produced products that were acceptable from a microbiological safety and a quality perspective. However, there was a problem with *E. coli* counts obtained from some of the products; *E. coli* counts in products were high during the summer season and decreased with relatively cooler weather. Some processors have purchase specifications based on *E. coli* counts for their products and meeting the specification could be difficult at times. It should be realized that not all *E. coli* cells are pathogenic.

Fresh catfish fillets were also screened for common and new and emerging pathogens. Products were obtained from the same processors and were analyzed at four different times of the year. In all, 120 samples were examined for each of the 11 pathogens, including *Campylobacter jejuni/coli*, *Escherichia coli* O157:H7, *Klebsiella pneumoniae* subsp. *pneumoniae*, *Plesiomonas shigelloides* and *Vibrio cholerae*. *P. shigelloides*, an emerging pathogen, and *V. cholerae* were isolated from the fresh catfish fillets. Frequency of isolation of *P. shigelloides* was very low and the pathogen was isolated only when the weather was warm; the isolation rate for *V. cholerae* was higher but was also isolated only during the warmer weather. Since these products are raw and are not consumed without cooking, the need for proper product handling after processing through the distribution system should be emphasized. Aquacultured products have a history of product safety and the combination of proper handling and cooking will prevent foodborne illness from these pathogens.

Fresh aquacultured catfish fillets were also surveyed for their antibiotic resistant bacteria during the various seasons. There was a significant difference in the number of antibiotic resistant bacteria which was attributed to the production source.

To improve the quality of the fresh catfish products, several mechanical and chemical processes were evaluated. High pressure spray washing has been evaluated using water at low temperature. Chemical agents (lactic, propionic and acetic acids) were added to water to increase shelf life of the products. Propionic acid was the best acidulant in reducing bacterial counts in broth cultures. However, during washing tests, all acids exhibited similar results by reducing bacterial counts by 1.0 log cycle.

L. monocytogenes does not appear to be very prevalent in aquaculture ponds or on whole catfish; contamination of fillets appears to occur post-harvest, and can be present on more than

30% of fillets.

Catfish fillets, prepared by harvesting and processing in an industrial setting at L & R Aqua catfish farms in Damon, TX, were treated by tumbling with 0.5, 1.0, 2.0, and 3.0% lactic acid for 1 or 3 min, followed by draining through placement of the fillet in a stainless steel perforated tub for 1 minute. Each group of treated fillets was placed in individual 100-L plastic bags and held in refrigerated storage at 4°C with ice surrounding the bags.

In general, lactic acid treatments extended the shelf life of catfish fillets 2-3 days compared to the control. Higher concentration lactic acid treatments imparted a discoloration to the fillets, but no detectable off-odors were noted. Total volatile nitrogen values remained constant at around 22-24 mg/100 g for all treatments. Trimethylamine values similarly remained unchanged during the shelf life study.

A procedure was developed and evaluated for a non-destructive method of sampling channel catfish for bacteriological analysis. The procedure is applicable for sampling of processed fish, fillets, frozen and breaded products. Benefits of the procedure include improved sensitivity of microbial detection, reduced time and cost of sample preparation for microbial analysis, and is non-destructive and easily accomplished. The procedure has been standardized to maximize the detection of microbes on processed catfish, and data are being prepared to seek approval of the procedure as an official method of microbial sampling of processed catfish. During the past year the procedure was evaluated for the detection of bacterial pathogens. Studies have demonstrated that the rinse technique can consistently recover as few as 5 viable *E. coli* O157:H7, 3 viable *S. typhimurium*, and 4 viable *L. monocytogenes* inoculated per catfish fillet. Because the procedure involves sampling the entire surface of the processed fish and includes two pathogen enrichment steps, it can detect low numbers of pathogens on the surface of catfish.

A commercially available one-day rapid *E. coli* enumeration test (EC Petrifilm 3M Company) was evaluated and compared to a standardized FDA procedure. Data revealed that the rapid test provides accurate counts in one-eighth the amount of time as compared to the FDA procedure. The rapid test will allow processors to ensure the microbial quality of their product prior to shipment to the public.

WORK PLANNED:

Objective completed.

IMPACTS:

Significant reductions in microbial populations could be obtained at processing facilities with alternative unit processing operations, such as the application of irradiation energy, the use of microbicidal chemicals in chilling waters, or temperature adjustments from harvest through product storage. The project has indicated that catfish products have pathogenic microbial profiles to prevent food borne illness in normal individuals. Also, pathogenic microbial profiles should permit international sales in most foreign countries.

The non-destructive procedure for sampling catfish is presently being used at the Fish Farming Center (Alabama Cooperative Extension Service) to evaluate catfish products from processing plants in Alabama. The rapid *E. coli* enumeration test will allow processors to ensure the microbial quality of their product prior to shipment to the general public and to demonstrate compliance with specific microbial specifications established by individual buyers in domestic and foreign markets.

Anti-listerial processing aids, such as Alta 2341, dehydroacetic acid, and nisin, can markedly lower levels of *L. monocytogenes* on catfish fillets. Ice impregnated with these antimicrobials reduces *L. monocytogenes* on catfish fillets and would incorporate easily into current retail practices.

Some improvement in shelf life of catfish fillets can be obtained through lactic acid treatment at processing. However, the benefit of increase in shelf life (2-3 days) would have to be weighed against increased processing costs to determine probable positive impacts of the treatment.

OBJECTIVE 3. CONDUCT A FOOD SAFETY HACCP AUDIT TO DETERMINE IF THIS APPROACH WOULD BE COST-EFFECTIVE AND RESULT IN INCREASED PRODUCT SAFETY.

ANTICIPATED BENEFITS:

The HACCP audit effort should provide the basis for the implementation of HACCP audits in individual plants. The audit form developed can be adopted or modified to fit individual needs.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

A HACCP audit sheet was developed and evaluated by a commercial processor in Mississippi. The audit sheet will be available to those who are interested.

IMPACTS:

The HACCP audit will become a quality assurance tool for the aquaculture processing industry and result in increased economic benefits in the future.

WORK PLANNED:

Objective completed.

OBJECTIVE 4. PRODUCE NEW PUBLICATIONS TO COMPLEMENT EXISTING PUBLICATIONS ON FOOD SAFETY AND SANITATION. THESE WOULD BE COMPLETED DURING THE SECOND AND THIRD YEARS OF THE PROJECT.

ANTICIPATED BENEFITS:

Information gathered for this project will be

a valuable tool for improving the microbiological safety of aquaculture products. This information will be given to state cooperative extension services to relay to the aquaculture industry because their knowledge of target industries and individuals within their state makes this method of education very effective. No other means are presently available to adequately relay this information than by state cooperative extension services.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Reports and relevant information from research scientists as well as a literature review dealing with the food safety/microbial study issues have been obtained. Representatives of both processor and consumer groups were interviewed; both groups agreed that negative media coverage has resulted in consumers perceiving that numerous fishery products are unsafe, and that there is a need for written materials to bolster consumers' confidence in aquacultured products.

Approximately 70 published articles, fact sheets, videos, and oral presentations at technical and consumer meetings have been produced during the course of the project. Information on foodborne illnesses due to microbiological contamination has been included in fact sheets and brochures. Valuable information including the purchasing, storage, handling, preparation, temperature control, spoilage and processing of aquacultured products has been produced. Publications have been written on pesticides, residues, risks, chemicals and regulators and their role in microbial food safety. All the information collected has produced substantial amounts of educational materials for the aquaculture industry and the general public.

WORK PLANNED:

Publications are planned for the research work which is to be completed during Year 3;

data from these studies will be given to the Extension publications project for development into fact sheets, brochures, and videos.

APPENDIX

PUBLICATIONS:

INPRINT

Perkins, B.E., Editor. 1993. *Proceedings: Aquaculture Products Safety Forum*. Auburn University, Marine Extension and Research Center. Mobile, AL. 157 pp.

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Ladewig, K., and D.W. Logan. 1992. You Can Do Catfish. SRAC Publication No. 501.

Ladewig, K., and D.W. Logan. 1993. El bagre es delicioso y nutritivo. SRAC Publication No. 501-S.

Bolton, L.F. 1993. Effects of antimicrobial agents and vacuum-skin packaging on shelf life of rainbow trout during refrigerated storage. M.S. Thesis, University of Georgia, Athens.

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Otwell, W. S. and V. Garrido. 1995. Total Quality Assurance (TQA) and Hazard Analysis and Critical Control Point: Manual for Clam Production and Processing. FL Sea Grant College Program, University of Florida, Gainesville, Technical Paper No. 80.

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fillets during the chilling process. In review.

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Otwell, S. 1994. Mandatory HACCP Programs for Processing Cultured Molluscan Shellfish. Annual Meeting of the Pacific Coast Oyster Growers Association, October 2-4, 1994. Seaside, OR.

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Wang, C., and M.L. Scruggs. 1994. An impedance method for evaluating the efficacy of lactic acid and pergenox to reduce bacterial counts in processed channel catfish. Animal Disease Research Workers in Southern States, March 28-30, 1994, Baton Rouge, LA.

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McCaskey, T.A. 1993. Bacterial evaluation of catfish products. Proceedings of the Aquaculture Products Safety Forum, February 2-4, Auburn University, AL.

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International Association Milk Food and Environ. Sanitation, Abstract #52, Atlanta, GA.

Anthony, B.K., F.A. Draughon, M.E. Denton, and W. Tan. 1993. Comparison of methods for isolation of *Listeria* from rainbow trout (*Oncorhynchus mykiss*). Proceedings, Annual Meeting International Association Milk Food and Environmental Sanitation, Abstract #143, Atlanta, GA.

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Huang, C.Y., Zheng, M., and Huang, Y.W. 1993. Psychrotrophic plate count, nucleotide degradation products and color changes of sodium lactate treated rainbow trout fillets as affected by packaging method at 4°C. Institute of Food Technologists Annual Meeting, July 10-14, Chicago, IL.

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Flick, G.J. 1994. Developing a total quality assurance program. Presented paper at the annual catfish processors meeting in Greenwood, MS. Sept. 1994.

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Mu, D.M. and Huang, Y.W. 1995. Effect of trisodium phosphate on *Listeria monocytogenes* attached to rainbow trout. Intnatl. Assocn. Food Milk and Environ. Sanitation Annual Meeting, July 30-August 2, Pittsburgh, PA.

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SUPPORT:

YEAR	SRAC FUNDING	OTHER SUPPORT				TOTAL OTHERSUPPORT	TOTAL SRAC+ OTHER SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER		
1	85,000	77,623				77,623	162,623
2	225,000	139,740	2,500	2,000	15,000	158,240	383,240
3	260,000	141,760	200	1,800		143,760	403,760
Total	570,000	359,123	2,700	3,800	15,000	379,623	949,623