

LOUISIANA AGRICULTURE

THE MAGAZINE OF THE LOUISIANA AGRICULTURAL EXPERIMENT STATION

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Aquaculture Issue

Cover: C.R. "Chuck" Weirich, a researcher at the Aquaculture Research Station, harvests catfish from one of his research ponds. His research associates are, left to right, Cliff O'Neal and Wei-Bing Yan. Photo by John Wozniak

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Photo by John Wozniak

At the Aquaculture Research Station, which is three miles from the LSU campus at Baton Rouge, there are more than 200 fish culture tanks, a fish hatchery, an aquaculture greenhouse and a 22,000 square-foot aquaculture research laboratory. These fish culture tanks are 12 feet in diameter.

Research facilities and support

The Aquaculture Research Station is one of 20 branch stations of the Louisiana Agricultural Experiment Station. The facility, which includes more than 200 fish culture tanks, a fish hatchery, an aquaculture greenhouse and a 22,000 square-foot aquaculture research laboratory, is three miles south of the LSU campus in Baton Rouge. The facility also includes 146 earthen ponds, ranging in size from 17 acres to 0.1 acre, that cover 100 surface acres of water. Crawfish aquaculture research is conducted at the Rice Research Station at Crowley in 55 earthen ponds with supporting laboratories. Other research programs are routinely conducted at commercial aquaculture farms. Aquaculture research is also part of the research program in seven academic departments including the School of Forestry, Wildlife and Fisheries; School of Veterinary Medicine; and the departments of Agricultural Economics and Agribusiness, Biological and Agricultural Engineering, Food Science, Civil and Environmental Engineering, and Veterinary Science.

Funding for aquaculture research comes from state and federal appropriations, state commodity boards such as the Louisiana Crawfish and Louisiana Catfish promotion and research boards, federal and state agencies, the Southern Regional Aquaculture Center and private sector grants and contracts.

To support aquaculture research, the Louisiana Cooperative Extension Service has two statewide aquaculture specialists and 10 area aquaculture and fisheries agents.

The School of Veterinary Medicine and Department of Veterinary Science have excellent laboratories to support research on aquatic animal diseases, and the school houses the Aquatic Animal Diagnostics Laboratory, a unit which provides disease treatment recommendations to the state's aquaculture producers at no charge. The Muscles Food Laboratory and the Agricultural Chemistry and Food Science departments support research on seafood safety and development of value-added products. ■



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Research and Extension Programs Address Needs of Louisiana's Aquaculture Industry

Robert P. Romaine

Even though aquaculture, or “farming of the waters,” has been practiced for centuries, it was more “art” than “science” until late into the 20th century. Today, the scientific principles used in traditional animal husbandry are applied to aquaculture, and new technologies to improve sustainability and profitability of fish farming enterprises are being developed rapidly. Aquacultural sciences, traditionally dominated by

grant institutions in allocating resources to research and extension programs in aquaculture.

With plentiful water, flat terrain and abundant coastal wetlands, Louisiana is a national leader in area and revenues derived from aquaculture. In 1998, nearly 3,600 aquaculture producers harvested more than 123 million pounds of cultivated aquatic animals with a farm value of \$152 million; value-added

(blue, channel, flathead), paddlefish and forage species (koi, golden shiner, bluegill) for stock enhancement of the state's recreational public water bodies.

Industry faces challenges

Louisiana's aquaculture industry is not without problems though. Several commodities are threatened by competition from foreign imports, environmental restrictions on land and water use, price increases in farm services and supplies, diseases associated with intensified production, off-flavor that hinders fish sales, bird depredation and difficulties in securing financing from commercial lenders. Continued growth and sustainability of the state's aquacultural enterprises depend on solutions from LSU Agricultural Center scientists being implemented by industry.

In 1966, to address the needs of the state's fledgling aquaculture industry, the Louisiana Agricultural Experiment Station (LAES) hired James W. Avault Jr. as a professor of aquaculture in the School of Forestry, Wildlife and Fisheries. Dr. Avault quickly initiated research programs that led to major changes and spurred growth in the state's crawfish farming industry. Research programs addressed the needs of the state's catfish farmers, and the potential for commercial development of other species in Louisiana was investigated, including marine shrimp, red drum, pompano, freshwater prawns, bait minnows, hybrid striped bass and tilapia.

In 1980, Dr. Avault wrote a report that laid the groundwork for a long-term program to enhance aquaculture research and technology transfer. Since then, aquacultural scientists were hired and research facilities upgraded and expanded. To bolster the effectiveness and efficiency of aquaculture research, LSU Agricultural Center administration created the Aquaculture Research Station on July 1, 1998. Its mission is to enhance



Photo by John Wozniak

The aquaculture research facility, which was built in 1991, became the Aquaculture Research Station in 1998. Robert Romaine was named its first resident director.

aquatic biologists, now include scientists from many disciplines, such as engineering, veterinary science, economics and food science. Their contributions have significantly increased the rate of development and adoption of new technologies. The LSU Agricultural Center has been a leader among land-

contributed \$99 million. Louisiana has among the most diversified aquaculture industries in the country led by channel catfish, crawfish, oysters and alligators followed by smaller commodities including bait minnows, soft-shell crabs and soft crawfish, hybrid striped bass, red drum, tilapia, gamefish fingerlings, turtles and ornamental fishes. State and federal fish hatcheries in Louisiana produce more than 6 million recreational game fish, such as largemouth bass, striped bass, hybrid striped bass, catfish

Robert P. Romaine, Resident Director and Professor, Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.

the competitiveness and profitability of the state's aquaculture producers and to promote the industry's continued economic development.

In 1999, the LAES has 15 aquaculture research projects involving 12 senior scientists and numerous support personnel. Most scientists have joint appointments with the LSU College of Agriculture to support teaching and graduate education programs. Four senior scientists on the LSU A&M campus direct aquaculture research, teaching and graduate education programs and work closely with Ag Center personnel. In 1999, scientists supervised 35 graduate students, including 16 working on doctoral degrees and 19 on master's degrees.

LSU Agricultural Center aquacultural scientists work closely with personnel in the Louisiana Department of Wildlife and Fisheries (LDWF) and the Louisiana Department of Agriculture and Forestry (LDAF). Early LAES research on mariculture was conducted at coastal aquaculture facilities operated by the LDWF. Collaborative LAES research programs in aquaculture have existed with scientists at regional universities including the University of Louisiana at Lafayette, Northwestern State University of Louisiana, Nicholls State University, Southern University and the Louisiana Universities Marine Consortium (LUMCON).

Aquaculture research and extension programs address many issues:

- developing management procedures for minimizing water use, effluent discharge and recycling water resources
- searching for genetic improvement of cultivated finfish and shellfish stocks through selective breeding, hybridization, polyploidy, sterilization and transgenic manipulation
- improving fish feeds for cost reduction and enhanced growth, and developing environmental friendly feeds that minimize nutrient release to the aquatic environment



Crawfish research is conducted at both the Aquaculture Research Station, shown here, and the Rice Research Station in Crowley.

- developing water management systems that limit water use, minimize waste discharge, minimize off-flavor in cultivated species and maintain a healthy environment of the cultivated fish, crustacean or mollusk
- developing vaccines and other management procedures that reduce the incidence and severity of diseases in cultivated aquatic animals
- investigating the potential for culture of non-indigenous species and their impact on native species
- improving production strategies and management programs to improve culture efficiency
- developing value-added products and improvements in product quality
- addressing economic sustainability of aquaculture in Louisiana

Louisiana has a unique opportunity to take advantage of continuing demand for fisheries products. Louisiana has the resources needed for aquaculture development, including highly fertile, flat agricultural lands conducive to pond development, a long growing season, abundant surface water and ground water

resources, and nearly 30 percent of the nation's coastal wetlands. Aquaculture integrates well into traditional agricultural operations and has potential to diversify business opportunities for Louisiana grain and animal producers.

Continued and expanded contributions of aquaculture to Louisiana's economic development require research designed to sustain economically viable businesses by increasing production efficiency through advances in nutrition, genetic improvement, water management and water use, disease control and prevention, production management, post-harvest product quality and development of value-added products. With the new millennium upon us, LSU Agricultural Center scientists look forward to the challenge of developing research, extension and education programs designed to improve the competitiveness and profitability of the state's aquaculture producers while providing comprehensive undergraduate and graduate curricula to develop quality aquaculture professionals. Further details on the LSU Agricultural Center's aquaculture research and educational programs can be viewed at our website: <http://www.agctr.lsu.edu/wwwac/research/aquaculture>. ■

Management of water quality and effluents from aquacultural systems

Profitable aquaculture depends on good water quality. Physiologically, aquatic animals respond more intensely to their environment than do terrestrial animals. The stress of poor water can lead to disease and poor nutrition and growth of cultivated aquatic animals.

LSU Agricultural Center scientists are addressing several issues related to water quality management in crawfish and channel catfish aquaculture. At the Rice Research Station in Crowley, scientists are studying the effects of long-term exposure of low oxygen on growth and mortality of red swamp crawfish. At the Aquaculture Research Station in Baton Rouge, management of algal populations for water quality improvement and off-flavor control in channel catfish ponds is being investigated in several projects which include polyculture with algal-feeding fishes, such as threadfin shad and tilapia; reducing odorous populations of blue-green algae by adding salt or trace minerals; and assessment of the partitioned aquaculture system.

In recent years, concerns about agriculture's effect on the environment, particularly related to non-point sources of pollution on stream and lake water, have been raised. The LSU Agricultural Center hosted a workshop in July of 1999 on "Agricultural Water Quality Issues for the 21st Century" to review, discuss and begin strategic planning on issues related to federally mandated requirements to protect the quality of Louisiana's surface water resources. The federal Clean Water Act of 1972 (formerly known as the Federal Water Pollution Control Act) and subsequent amendments enacted in the past decade require that the nation's streams, rivers and lakes be sufficiently clean to be fishable and swimmable by a target date near the year 2010. Enforcement of the Clean Water Act is given to the individual states by the U.S. Environmental Protection Agency.

Effluents from aquacultural operations are released into streams and rivers during heavy rainfall, when harvesting fish, to accommodate reproductive cycles of the cultured animal (for example, draining crawfish ponds to stimulate burrowing) or to maintain acceptable water quality in the culture system. Many of the state's aquacultural producers, particularly crawfish farmers, use streams and rivers as their principal water source.

In the 1970s, most states did little to regulate aquacultural effluent discharge, mostly because aquaculture was considered too small an industry to have a significant impact on the environment. But growth in the nation's aquaculture industry, coupled with provisions of the federal Clean Water Action Plan of 1998 and legal action against the EPA by non-governmental

organizations, necessitates that Best Management Practices (BMPs) for agricultural commodities, including aquaculture, be developed and implemented. Failure to address these needs could constrain future growth and development of Louisiana's aquaculture industry.

In April of 1999, a three-year regional project on "Management of Aquacultural Effluents from Ponds" was funded by the Southern Regional Aquaculture Center (SRAC). This study follows a three-year project completed in 1994, also funded by SRAC, in which 16 scientists representing 10 universities, including the LSU Ag Center, cooperated to characterize effluents from commercial aquaculture operations in the South, including commercial crawfish farms in Louisiana.

The present study, which includes participation of aquacultural scientists from seven universities and one state agency, will provide more detailed data on effluent quality and quantity from major aquacultural industries in the South, including catfish, crawfish, bait fish, hybrid striped bass and marine shrimp. Emphasis will be placed on determining the amount of suspended solids and phosphorus discharged during intentional (for example, draining at harvest) and non-intentional (storage overflow during rainfall) effluent releases and their impact on receiving waters. Based on existing information, supplemented by the project findings, a comprehensive set of general BMPs that can be implemented to reduce the environmental effects of pond aquaculture will be developed. Supplemental BMPs for various pond cultured species in the southern region will be formulated, too. As in the previous study, LSU Ag Center scientists will have responsibility for crawfish aquaculture.

From data generated to date, the best approach to managing effluents from aquaculture is (1) to reduce the amount of waste produced in the pond by using high quality feed and efficient feeding practices and (2) to decrease the volume of water discharged by minimizing water exchange, reusing water for several production cycles and maintaining enough storage volume in ponds to prevent rainfall overflow.

Louisiana aquaculture producers can be assured that Ag Center scientists will continue research and extension programs to develop practical, cost-effective solutions to management of pond effluents to comply with federal and state regulatory requirements, protect the environment and sustain profitability. ■

Robert P. Romaine, Resident Director and Professor, Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.

Aquaculture Development: Potential for Growth in the New Millennium

James W. Avault Jr.

Aquaculture is evolving world-wide because of a shortfall of fishery products from oceans and inland waters. After World War II, it appeared that the world's fisheries resources were virtually unlimited. World landings during 1948 to 1952 averaged 21.9 million metric tons per year and rose steadily until 1968, when increases continued but at a slower rate. By the early 1990s, global fisheries harvest plateaued near 100 million metric tons. Today, much of the catch has shifted from valuable species, such as flounder, haddock, Atlantic cod and swordfish, to much less desirable species, such as spiny dogfish, skate and shark. About 30 percent of the world's fisheries harvest is reduced to fish meal and oil. One might think the vast oceans would be our salvation with respect to food problems, but 90 percent of our oceans are biological deserts.

Although the fishery harvest from the world's oceans has stagnated, world population growth and per capita consumption are dynamic. About 90 million people are added to the world's population each year. By 2025, the population is forecast to reach 8.5 billion. World per capita consumption of seafood is 28.6 pounds per year. With no change in per capita consumption, the world's population will need more than 55 million metric tons per year of seafood by 2025. The wild fisheries may be able to produce a bit more with better management, but the bulk will have to come from aquaculture.

Aquaculture dates to about 2000 B.C. in China when carp were raised along with the manufacture of silk. The silkworm pupae and feces were used to feed fish. For centuries, aquaculture provided food mostly at a subsistence level. After World War II, some developing nations concentrated on farming staple aquatic species for animal protein and profit. Various carp and tilapia species were farmed.



James Avault, professor emeritus, is the "father" of aquaculture research at the LSU Ag Center. He was hired in 1966 to develop a research program.

In the 1980s, many nations, both developed and developing, began shifting attention to aquatic species of high value, such as marine shrimp and salmon. Developed nations, such as the United States, Japan and those in western Europe, saw demand for high-value species escalate. Developing nations saw the possibility of foreign exchange with affluent countries.

Records on world aquaculture production appeared around 1966 at which time the Food and Agricultural Organization (FAO) of the United Nations estimated production at 1.1 million metric tons, or about 3 percent of the world fishery production. Over the years aquaculture production has grown nearly 8 percent annually. By 1995, the FAO estimated world aquaculture production (excluding cultivated seaweeds) near 28 million metric tons, nearly 20 percent of the world fishery production, with a wholesale value of \$42.3 billion. The FAO estimates, based on demographic population growth alone and no

increase in per capita consumption, that global aquaculture will supply 35 percent to 40 percent of world seafood supply by 2010 and 50 percent to 60 percent by 2025.

Imports to the United States have reached record levels of nearly \$8 billion. The United States is also a major aquaculture producer. Channel catfish dominates production. In 1998, 560 million pounds were produced with a value of about \$415 million. The four major catfish-producing states are Mississippi, Arkansas, Alabama and Louisiana.

Other important species cultivated in this country include rainbow trout (57 million pounds); crawfish (40 million to 50 million pounds); tilapia (18 million pounds); salmon (33 million pounds); marine shrimp (6 million pounds); mollusks, primarily oysters

*James W. Avault Jr., Professor Emeritus,
Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.*

(24 million pounds); and a myriad of other species, such as hybrid striped bass, bait fishes and ornamental fishes.

Fastest growing segment in agriculture

According to the U.S. Department of Agriculture, aquaculture is the fastest growing segment of the nation's agriculture, increasing at an annual rate of about 8 percent per year. Tilapia production alone has increased 300 percent in the past five years. Moreover, tilapia consumption in the United States increased 26 percent from 1996 to 1997.

The LSU Agricultural Center began exploring the niche of aquaculture around 1965. Scientists initiated a research program through the Louisiana Agricultural Experiment Station (LAES). Meanwhile, the graduate program in the School of Forestry, Wildlife and Fisheries began adding aquaculture courses, and shortly thereafter the Louisiana Cooperative Extension Service (LCES) added a wildlife and fisheries specialist and an aquaculture program. At this time token acreage was in crawfish production, and catfish farming was generating considerable interest. Early research by the LAES focused on the basics of farming crawfish and catfish. For years the crawfish industry was based on hit or miss with the wild crop from the Atchafalaya River Basin. Significant crawfish crops were produced three out of five years. Some years there was a bumper crop; some years virtually nothing. Through research, the LAES researchers developed methods for predictable farm-raised crops. With this came the establishment of markets other than the traditional family weekend crawfish boil. Eventually, overseas markets were established.

Over the years, the LSU Agricultural Center, responding to the needs of the nascent industry, developed a cultivated forage crop food-delivery system to feed crawfish. With rice as a forage instead of formulated feeds, the cost of farming crawfish went down and profits up. Networking with LCES personnel and crawfish farmers, LAES scientists developed methods of double-cropping rice and crawfish, developed manufactured baits that evolved into a multimillion dollar industry, established guidelines for managing water quality, designed new traps and trapping programs for harvesting, and in general responded to the needs of crawfish farmers.

Early catfish industry

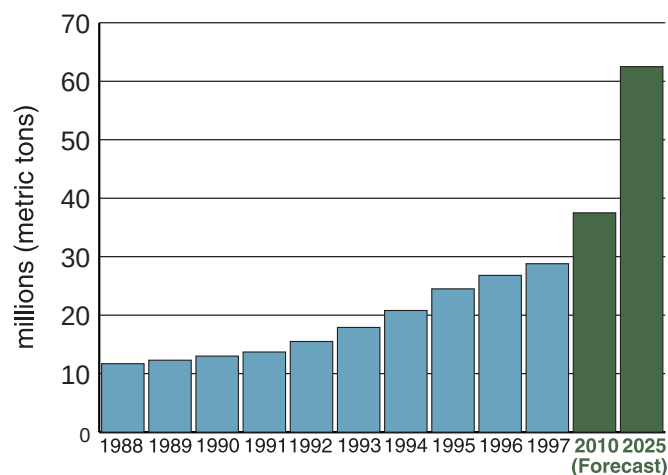
The early catfish farming industry was viewed by the LSU Agricultural Center in the same manner. For example, a number of farmers hit brackish water when drilling water wells. LAES personnel working with scientists at the Louisiana Department of Wildlife and Fisheries' Rockefeller Wildlife Refuge learned that catfish not only tolerate slightly brackish water, they thrive in it. Catfish, the studies showed, could be grown in waters up to 8 parts per thousand salinity. Moreover, these catfish never had off-flavor or brown-blood disease caused by nitrite toxicity. Further research showed that a dreaded disease called white spot disease or "Ich" was controlled when fingerlings were held in salinity for a week. With continuous aeration and high feeding rates, more than 6 tons of catfish were produced per acre each season.

No other state has the potential for aquaculture as does Louisiana. It has all

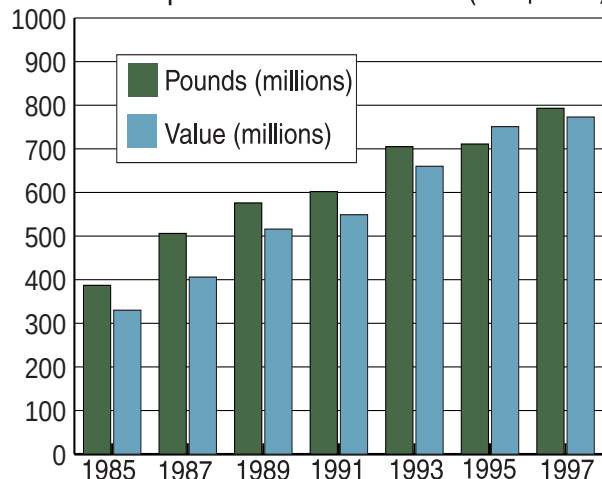
the essential prerequisites: (1) people with a heritage in fisheries, (2) a long growing season, (3) an established aquaculture industry well integrated into traditional agriculture, (4) abundant land well suited for aquaculture, (5) an abundance of both surface water and ground water, (6) LSU Agricultural Center personnel with expertise in aquaculture research and technology transfer and (7) people with a reputation for good cooking and a fondness for quality seafood.

In 1986, the LSU Agricultural Center undertook a two-year, statewide investigation of the potential for aquaculture in Louisiana. The study included an evaluation of soils and topography, climate, water resources, seafood processing capabilities, transportation systems and, in general, the infrastructure required to develop an aquaculture industry. Superimposed on these findings was the suitability of each parish for the culture of nine aquatic species, including catfish and crawfish. The report concluded that the potential for aquaculture development exists in every parish. More than 21 million acres, 75 percent of the land area in Louisiana, is sufficiently level and has sufficient water-retaining ability to allow leveed pond construction. Adequate supplies of ground water and surface water are available in most areas. Louisiana has potential to become the seafood market of the nation. Through the combined efforts of LSU Agricultural Center research and extension programs, and governmental leadership, the citizens of Louisiana may reap the monetary and employment benefits of an expanded aquaculture industry. ■

World Aquaculture Production



U.S. Aquaculture Production (All Species)



The LSU Agricultural Center's aquatic animal nutrition program conducts research on the development and improvement of feeds for aquatic animals of commercial importance in Louisiana. These include channel catfish, hybrid striped bass and tilapia. Robert C. Reigh is in charge of this area of research.

Photo by John Wozniak



Nutrition Research Reduces Catfish Production Costs and Improves Pond Environment

Robert C. Reigh

The art of feeding fish is thousands of years old, but the science of fish nutrition began only about 50 years ago. Fish nutritionists, like their counterparts in the animal and poultry sciences, seek to determine the dietary requirements of fishes and other aquatic animals cultured for human use and to develop quality feeds for the numerous species produced under highly controlled conditions.

The LSU Agricultural Center's aquatic animal nutrition program conducts research on the development and improvement of feeds for aquatic animals of commercial importance in Louisiana. These include channel catfish, hybrid striped bass and tilapia.

In catfish farming, feed cost typically constitutes half the cost of production. With such high feed expenditures, even a small reduction in feed price can provide substantial savings.

Protein reduction

One way to reduce the cost of fish feed is to reduce its protein content,

which is often the most expensive part. Fish feeds tend to contain relatively high levels of protein (usually 25 percent to 45 percent of diet weight) because the species produced in most commercial facilities grow well on such diets. But research with a number of fish species has shown that dietary protein levels can be reduced in properly balanced diets without negative effects on growth or body composition. The magnitude of the protein reduction depends on the dietary requirements of the species and the nutritional balance (energy and amino acid composition) of the diet.

Channel catfish are omnivorous in their natural food habits and will accept a wide variety of ingredients in formulated diets. This makes feeding catfish less expensive than feeding carnivorous species. Catfish producers can substitute lower cost plant products, such as soybean meal and cottonseed meal, for the higher cost animal protein supplements, such as fishmeal and meat and bone meal, which typically constitute a

large portion of the diet of cultured carnivorous fishes. Because catfish can use relatively high levels of dietary carbohydrate, which is often poorly digested by many other fishes, large quantities of inexpensive, starchy grains, such as corn and wheat middlings, can be incorporated in catfish diets to reduce cost.

Plant vs. animal diets

Recent research with catfish has demonstrated that balanced, all-plant protein diets can produce weight gains equal to those obtained with high-animal protein (8 percent fishmeal) diets, which were the industry standard just a few years ago. In a three-year study at the LSU Agricultural Center's Aquaculture Research Station, yields of pond-raised

Robert C. Reigh, Professor, Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.

catfish fed an all-plant protein diet (3,257 pounds per acre per year) did not differ significantly from yields obtained with a diet containing animal protein (3,103 pounds per acre per year). In addition to lowering feed cost, the all-plant protein diet provided an added benefit of reducing the fat content of harvested fish.

Other studies at the station are investigating the optimum amino acid balance for channel catfish diets. Proteins, which are constructed of chains of amino acids, differ in their nutritional value for fish based on the types and quantities of amino acids they contain. Proteins from ingredients of animal origin, like fishmeal, tend to be better amino acid sources for fish than proteins of plant origin, which are often deficient in one or more essential amino acids. Nonetheless, deficiencies in single ingredients can be overcome by combining ingredients to satisfy dietary amino acid requirements. This approach has been taken in a three-year production study to determine the effect of reduced levels of certain dietary non-essential amino acids on catfish growth. Results of the study, in its third and final year, are not yet available, but weight gains of fish fed the experimental diets in a nine-

month laboratory growth trial (Figure 1) indicate that protein levels below 29 percent do not reduce catfish growth if dietary amino acid composition is balanced properly.

Digestibility affects environment

Digestibility of fish feeds has become an environmental issue. Nutrients in uneaten and undigested feed act to fertilize ponds and can stimulate algal blooms and other negative environmental effects if concentrations are sufficiently high.

Phosphorus is usually the most limiting nutrient for aquatic plant growth. It is relatively abundant in fish feeds, and much of it is present in a chemical form (phytic acid) that cannot be digested by fish or other simple-stomached animals. Phosphorus that passes through the fish's digestive system is eventually released into the pond water by decomposition, where it stimulates the growth of algae and other vegetation.

The most effective way to limit over-fertilization of ponds and the management problems that excessive nutrient loads cause is to reduce the

quantity of nutrients entering the system. A project under way at the Aquaculture Research Station is evaluating the feasibility of adding phytase enzyme to catfish diets to release the bound phosphorus in phytic acid so it can be absorbed in the digestive process. Results to date indicate that a concentration of 500 units of phytase per kilogram of diet significantly increases uptake of dietary phosphorus by channel catfish fed an all-plant diet, thereby decreasing the quantity of phosphorus entering the pond environment and reducing the need for dietary phosphorus supplements.

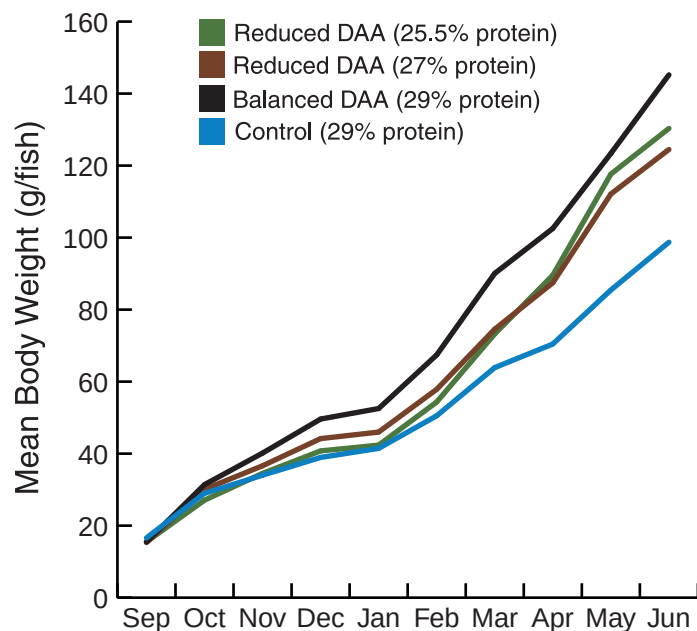
Reducing particle size

Digestive efficiency also is affected by the size of particles passing through the digestive tract. As food particles move through the gut, enzymes break down proteins, fats and carbohydrates exposed on their surfaces. Because small particles have a greater surface-to-volume ratio than large particles, digestion efficiency should be greater for a given weight of small particles than for an equal weight of large particles. Work in progress at the research station involves quantifying the effect of feed-ingredient particle size on digestibility of protein, energy and dry matter (total organic and inorganic matter) in channel catfish diets. The goal is to quantify changes in digestibility across the range of particle sizes to determine the point at which further reduction in particle size no longer improves digestibility. Application of the results of this research could help to produce diets that are used more efficiently and that provide better conversion of feed to weight gain.

Good pond environment

A good pond environment is essential for fast-growing, healthy fish. At high stocking densities (up to 10,000 fish per acre) and heavy feeding rates (up to 120 pounds of feed per acre per day), maintaining good water quality can become a management challenge. Use of diets with reduced protein content, improved amino acid profiles, greater organic matter digestibility and increased phosphorus availability will result in less metabolic waste, organic matter and phosphorus entering catfish ponds, which in turn will decrease oxygen demand from decomposition processes and reduce problematic algal blooms. Thus, improved diets offer both economic and environmental benefits to catfish producers. ■

Figure 1. Body weights of channel catfish fed diets containing different levels of protein and dispensable amino acids (DAA). The control diet was a typical catfish formulation. The balanced DAA (29 percent protein) diet had similar amino acid composition to catfish tissue. The reduced DAA (27 percent and 25 percent protein) diets had amino acid concentrations of 10 percent and 20 percent, respectively, below that of the balanced DAA diet. Fish were held from September to June in tanks that received flow-through, ambient temperature water from a pond.



Improved Control of Channel Catfish Spawning

Terrence R. Tiersch

For more than seven decades, catfish farmers have relied on the warming of spring to trigger spawning in channel catfish. The necessary water temperatures (75 degrees F to 85 degrees F) for

spawning last only a month or two, typically May and June in southern Louisiana, making this a busy time. The farm-raised catfish industry has adapted to this through specialization. Rather

than spawn their own fish, most farmers purchase stocking fish, called fingerlings, from suppliers.

Researchers at the LSU Agricultural Center's Aquaculture Research Station have been working for the past three years to lengthen and control the catfish spawning season. This work, supported in part by the Louisiana Catfish Promotion and Research Board, involves adding warm water to small ponds (0.1-acre) that contain broodstock channel catfish. By raising the pond temperature, researchers have been able to spawn catfish as early as March, doubling the length of the spawning season. Maintaining ponds within the proper temperature zone can avoid temporary shutdowns of spawning caused by cold snaps. Warming small broodstock ponds early in the spring would produce fry, the newly hatched catfish, that could be raised in the warm ponds to yield large fingerlings early in the season. The increased production of fry and fingerlings could prevent the shortages that have hampered the stocking of catfish in the past.

Another benefit of controlling the spawning season is disease prevention. Before catfish reach about 3 inches in length, they are susceptible to diseases including enteric septicemia, a bacterial disease that can destroy stocks of fingerlings. Because this disease proliferates at temperatures above 85 degrees F, it could be controlled by maintaining temperatures in the fry and fingerling ponds (used after the broodstock are removed) at about 80 degrees F.

Artificial spawning

Spawning procedures have remained essentially unchanged since the 1920s. Containers are placed into ponds for the catfish to enter and spawn, simulating the natural situation in which channel catfish would spawn in hollow logs or holes in riverbanks. Farmers collect the egg masses from the cans and bring them into the hatchery for fry

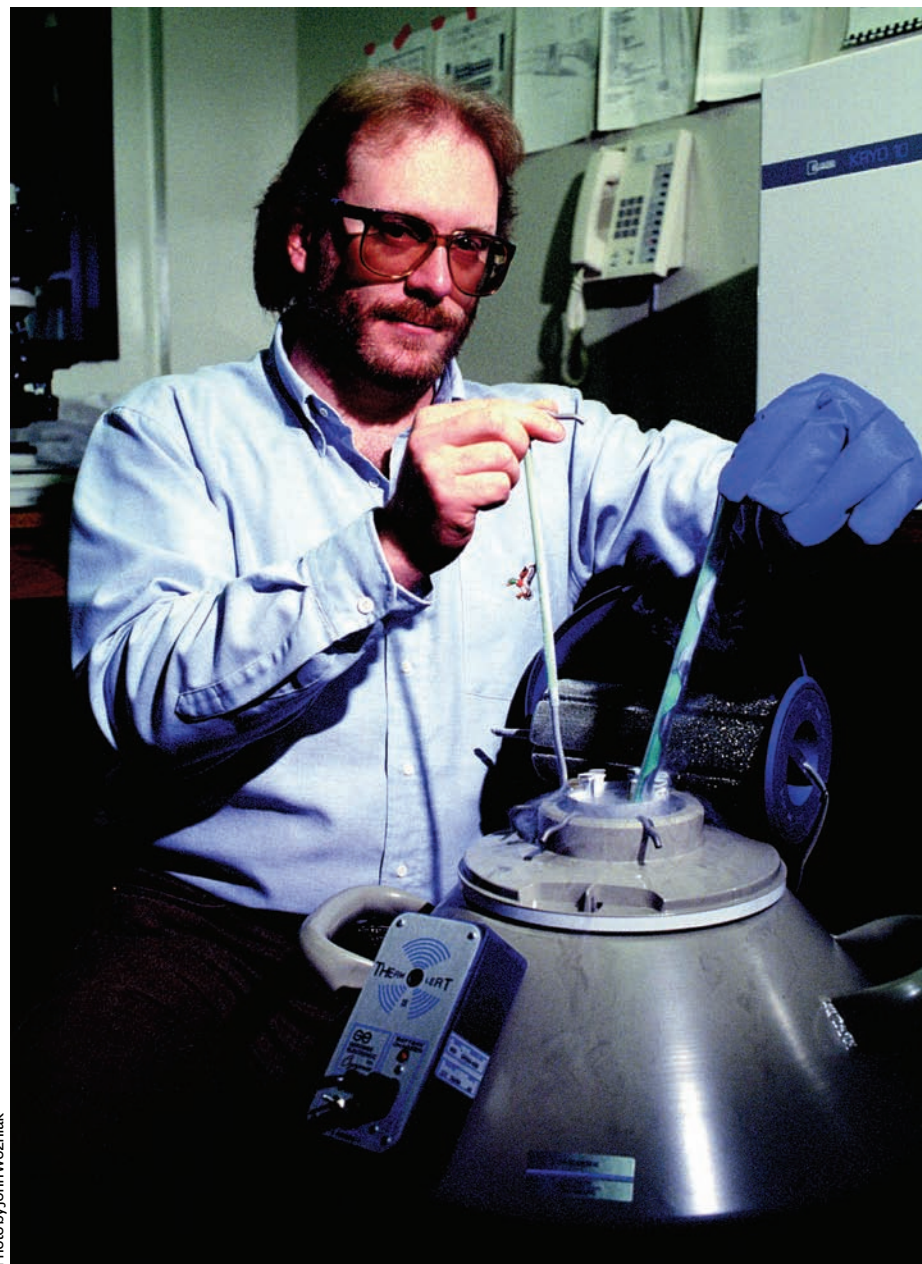
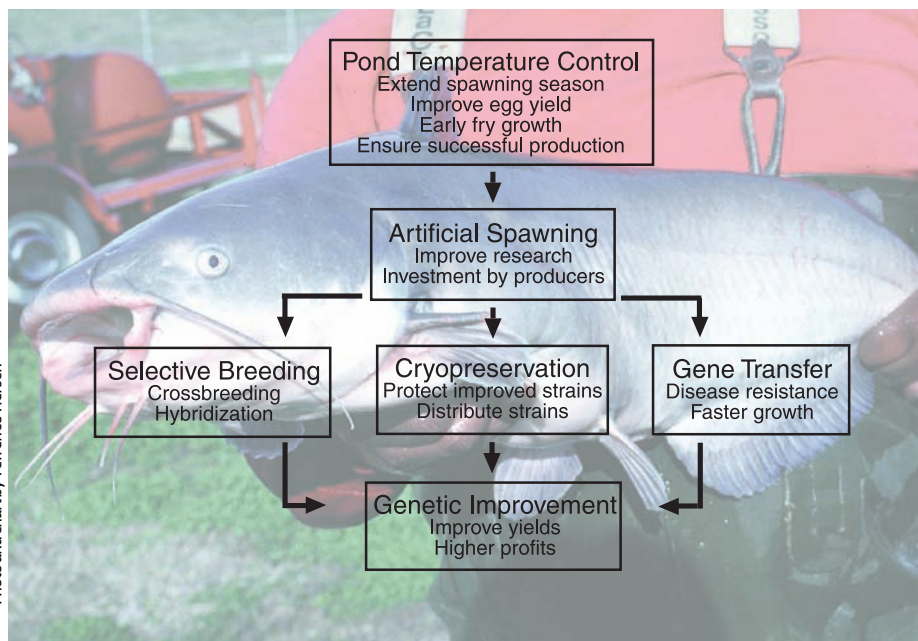


Photo by John Wozniak

Another important component for genetic improvement, already available for application, is sperm cryopreservation. In 1991, while at the U.S. Department of Agriculture, Terrence R. Tiersch produced the world's first channel catfish from thawed sperm, and the Aquaculture Research Station has developed cryopreservation procedures for sperm of more than 30 species of fish and shellfish since then. The station also was first in the successful production of a cultured aquatic food species (oysters) from frozen larvae.

Terrence R. Tiersch, Professor, Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.



production. This procedure, although effective for producing large numbers of fish, greatly hampers genetic improvement because it allows only minimal opportunity for selective breeding.

Researchers at the Aquaculture Research Station have improved methods to spawn catfish artificially in the laboratory. At present this technique is limited to use as a research tool because of cost, complexity and dependability. But the technique has potential to move the catfish industry into the era of genetic improvement. Artificial spawning involves large-scale collection of unfertilized eggs from females to allow controlled fertilization with sperm from specific (superior) males. Fingerling producers could specialize in this technology, which is used for most other farmed fishes, and produce a higher quality fish for sale to farmers. The extra expense would be offset by the superior performance of the improved stocks.

Developing hybrids

Another advantage of artificial spawning is the ability to develop hybrids. Consider, for instance, the hybrid produced by crossing the female channel catfish with the male blue catfish. This hybrid is considered superior to both parental species in disease resistance, tolerance to low oxygen and uniformity of meat yield. This valuable hybrid is not readily available to producers, however, because the two species do not naturally interbreed. Artificial spawning could produce hybrid fingerlings for the Louisiana catfish industry. The availability of

genetically superior catfish would make Louisiana catfish producers more competitive with the larger operations of other states. Raising such fingerlings in heated ponds would round out this picture with a larger, superior fish available for stocking.

Freezing sperm

Another important component for genetic improvement, already available for application, is sperm cryopreservation. In 1991, the author produced the world's first channel catfish from thawed sperm. Since then, the Aquaculture Research Station has developed cryopreservation procedures for sperm of more than 30 species of fish and shellfish. The station also was first in the successful production of a cultured aquatic food species (oysters) from frozen larvae.

Cryopreservation offers many benefits for the catfish industry. Frozen sperm can ensure that particular traits can be reliably produced over extended periods. Sperm from valuable males could be used for years, even long after their death, and could be used to fertilize eggs from many different females in a given year. This would provide the groundwork for selective breeding programs. Storage of frozen sperm is considerably cheaper and, in many ways, safer than maintaining broodstock in ponds, which is expensive and takes up space that could be used for production. Maintaining a large number of different strains of broodstock is prohibitively costly, even to large-scale fingerling producers.

In addition, there are many ways to lose valuable catfish broodstock. Some sources of loss can be quite rapid, such as disease outbreaks or low oxygen levels, but broodstock loss can be gradual, too. The insidious loss of genetic integrity by inbreeding or outbreeding depression, for example, can result in poor growth or reproduction.

Clearly, broodstock maintenance can pose significant problems, yet successful genetic improvement of catfish will require the availability of numerous different genetic stocks. Similar problems have been addressed in other agricultural industries such as beef, poultry and pork by freezing sperm. This technology offers obvious advantages in the preservation of desirable genes, in selective breeding, cross-breeding and hybridization, and in standardization of broodstock quality, although it remains unavailable without the use of artificial spawning.

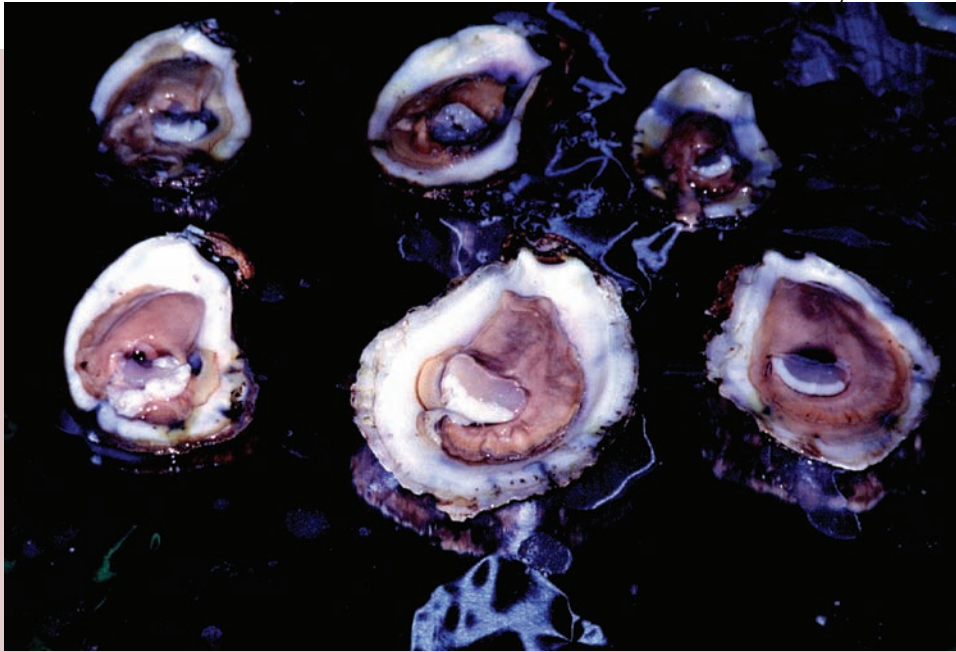
More control

Temperature control of ponds is an important component in an overall program to make artificial spawning and genetic improvement available to the Louisiana catfish industry. Our research offers a glimpse into the future in which catfish farming is more controlled by the farmer and more like the predictable production systems already in place in crop and livestock agriculture.

The future needs of the channel catfish industry in the coming century are impossible to forecast. The future could bring problems quite different from those of today. New diseases, changes in production systems or different market structures could require specific characteristics of catfish not valued at present. Storage of sperm from numerous stocks could ensure a reservoir of genetic material available for specific genetic improvement. By developing reliable, simple and inexpensive methods for artificial spawning, the Louisiana catfish industry can assume a leading role in genetic improvement and hybridization of catfish and move in step with the beef, pork and poultry industries in protecting, developing and distributing genetic improvements. ■

Acknowledgments

Personnel involved in catfish farming research include Mark Bates, John Buchanan, Michael Christensen, Donald Glenn, Robert Lang, Nyanti Lee, Carmen Paniagua-Chavez, Greg Roppolo, William Wayman and Gang Yu.



Adult oysters are good candidates for transgenic research because they do not move and thus are easily monitored. Research on gene transfer in oysters is part of a comprehensive program focusing on problems facing the oyster industry.

Gene transfer in oysters

One problem facing Louisiana's oyster industry is disease. The major culprits are the protozoans *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX). In the Gulf of Mexico, it is estimated that Dermo infection kills more than half the adult oyster population. Dermo and MSX have both been significant factors in the recent collapse of the oyster fishery in Chesapeake Bay. The transfer of human pathogens, such as *Vibrio vulnificus* and *V. cholera*, from oysters to humans has become a serious concern as well.

Transgenic technology may offer solutions to these problems. One focus of transgenic research at the LSU Agricultural Center is use of reporter genes. These genes produce easily recognizable changes upon successful transfer and subsequent expression. We used a gene known as green fluorescent protein, isolated from a jellyfish, as a reporter. Oysters successfully expressing this gene produce a protein that will emit a bright green when exposed to fluorescent light. We have developed techniques for gene transfer in oyster gametes and embryos and have observed expression of green fluorescent protein in oyster larvae. We also have delivered this gene and observed expression of green fluorescence in the blood cells of adult oysters. Positive results for gene transfer with this reporter gene indicate that our techniques are effective and allow for improvement of gene transfer efficiency. Other genes of interest can be transferred with these techniques to improve disease resistance in oysters.

Research at the LSU Ag Center, conducted with support from the Louisiana Sea Grant Program, has identified proteins, known as lytic peptides, that are toxic to Dermo and *Vibrio vulnificus*. In our laboratory, we have cloned genes for the lytic

peptide cecropin B as well as the synthetic lytic peptide phor 21. These genes are used also in transgenic modification of channel catfish for increased disease resistance (see page 14). Delivery of genes such as these may lead to the production of an oyster capable of eliminating pathogens.

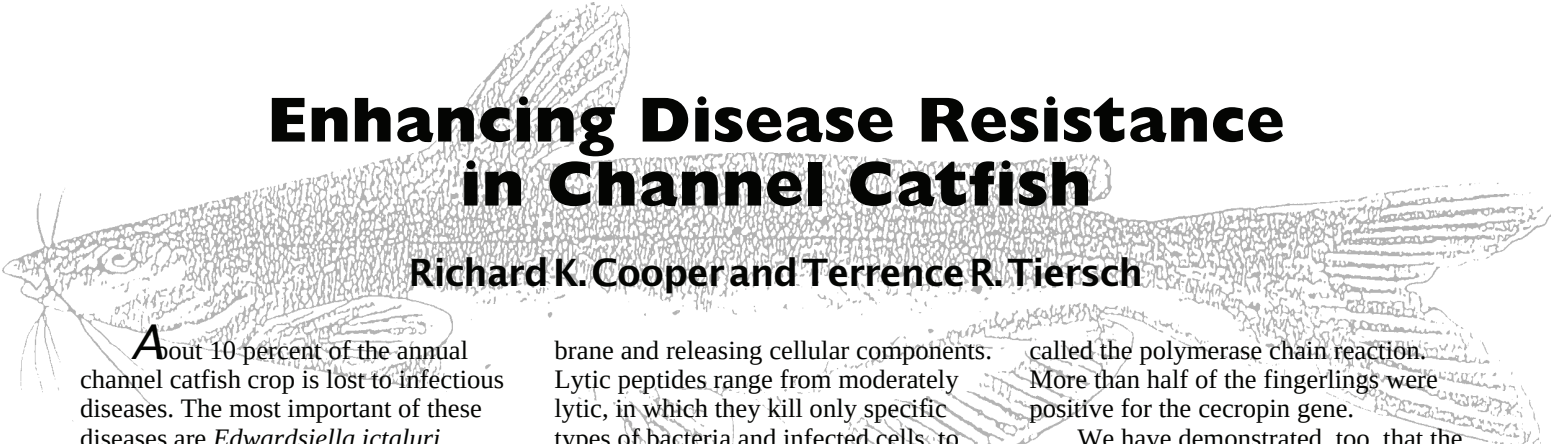
To prevent the accidental release of genetically modified organisms, we have developed techniques for working with oysters in a completely controlled environment using artificial seawater at some distance from the coast. Adult oysters are good candidates for transgenic research because they do not move and thus are easily monitored. This research is part of a comprehensive program focusing on problems facing the oyster industry.

Other research includes oyster chromosomes and physical genome mapping, sterilization and ploidy manipulation, hatchery technology, cryopreservation, artificial spawning, recirculating system techniques, cell culture, cryopreservation of gametes and larvae, pathogen biology, and oyster immune system function and defense mechanisms. Through our research, the potential exists for commercial production of sterile, genetically modified oysters for improved culture. ■

Acknowledgments

Personnel involved in oyster gene transfer research include Philip Cheng, Jerome LaPeyre, Karen McDonough, Amy Nickens, Brandye Smith and Quiyang Zhang.

John T. Buchanan, Doctoral Candidate, Department of Oceanography and Coastal Sciences; Terrence R. Tiersch, Professor, Aquaculture Research Station; and Richard K. Cooper, Associate Professor, Department of Veterinary Microbiology and Parasitology, LSU Agricultural Center, Baton Rouge, La.



Enhancing Disease Resistance in Channel Catfish

Richard K. Cooper and Terrence R. Tiersch

About 10 percent of the annual channel catfish crop is lost to infectious diseases. The most important of these diseases are *Edwardsiella ictaluri*, *Flavobacterium columnare* and channel catfish virus. Only the antibiotics Romet and oxytetracycline, which can be incorporated into feed, are approved by the Food and Drug Administration (FDA) to treat *E. ictaluri* and *F. columnare*, and there is no treatment for channel catfish virus. The farmer has to rely on good management to prevent outbreaks of disease. Even the most carefully managed pond can experience disease problems, however.

With treatment options limited, much emphasis in the past several years has been placed on vaccine development, with the most progress to date being made on *E. ictaluri* and channel catfish virus. Although vaccines hold promise, the drawback is that a vaccine has to be made for each disease-causing organism, and each has to be approved by the USDA. The entire process takes several years.

Several years ago, we began to investigate other ways to protect catfish against these diseases. Our goal is to produce a fish that alone can withstand infection or have its immune system further strengthened with vaccines to specific pathogens as the vaccines become available.

To accomplish the goal of enhancing the catfish immune system, we chose a gene for a lytic peptide that is controlled by an acute phase promoter, which can be compared to an on/off switch. Lytic peptides are small peptide molecules produced by a wide array of animals from invertebrates to mammals, including humans. There are many classes of lytic peptides based on their structure, but most share a common component. They are part of the first line of defense for an organism, and they kill pathogens by destroying the cell mem-

brane and releasing cellular components. Lytic peptides range from moderately lytic, in which they kill only specific types of bacteria and infected cells, to the very lytic. Melittin, for example, is the major component of honeybee venom. We chose the lytic peptide cecropin B from the giant silk moth, *Hyalophora cecropia*, because it kills a wide range of Gram negative and Gram positive bacteria without damaging host cells. Previous work had shown cecropin B to be very effective in killing *E. ictaluri*, one of the primary pathogens against which we wanted to protect channel catfish.

Another key component of the cecropin B gene is the acute phase promoter (APR) that controls expression of the peptide. A promoter controls when a gene will be "turned on." There are basically two types of promoters: those that are expressed, or on, at some level all the time, and those that are turned on only when needed. The APR promoter is "off" when it is not needed (when no pathogen is present), but it can be turned on quickly by molecules in the immune system that are responding to an invasion by a pathogen. This system allows the catfish to express the cecropin B peptide when a pathogen is encountered and not expend unneeded energy when there is no pathogen.

To deliver the cecropin B gene to the catfish genome, we developed a system to optimize DNA incorporation (see page 25). Using a technique called electroporation, in which a small electrical charge is applied to the cells that momentarily creates pores in the cell membrane to allow the DNA to enter the cell, we transferred the plasmid vector containing the cecropin B gene to unfertilized channel catfish eggs. The eggs were fertilized using artificial spawning techniques developed in our laboratory, hatched and allowed to grow in a recirculating tank system housed in a laboratory approved for transgenic research.

When the fish resulting from the experiment were large enough to handle, a small sample of blood was taken from each fish and analyzed for the presence of the cecropin B gene using a technique

called the polymerase chain reaction. More than half of the fingerlings were positive for the cecropin gene.

We have demonstrated, too, that the APR promoter functions in the manner intended. None of the cecropin B product is detectable in healthy, transgenic fish, but it is detectable 12 hours after the fish has been exposed to *E. ictaluri*.

To demonstrate whether or not cecropin B could protect transgenic catfish against *E. ictaluri*, we exposed an equal number of transgenic and non-transgenic fish with a virulent strain of the bacterium. Three days after infection, the fish were cultured for *E. ictaluri*. The non-transgenic, or control fish, had very high levels of bacteria in the hind kidney; in the transgenic fish, 50 percent of the fish had no detectable levels of bacteria, 34 percent had greatly reduced levels and the remaining 14 percent were no different from the non-transgenic fish. The differences in protection against *E. ictaluri* may be caused by the number of copies of the cecropin gene that inserted into the catfish chromosome. More copies could mean higher levels of lytic peptide resulting in more rapid clearance of the bacterium. We have shown that the use of gene transfer in channel catfish is feasible and that protection against *E. ictaluri* is possible.

Our long-term goal is to provide the catfish industry of Louisiana with a superior strain of channel catfish that has the ability to fight infection against the common diseases now plaguing the industry. This can be accomplished with an animal carrying a gene such as cecropin B and live attenuated vaccines as they become available. One key feature of having these animals available, however, is to produce a fish that is sterile. Recently, we have begun to pursue avenues to induce sterility (see page 16). ■

Acknowledgments

Funding support came from the USDA and the Louisiana Catfish Promotion and Research Board. Personnel involved included Brandye Smith, Jan Lousteau, Jackie McManus, Mark Bates, Greg Roppolo, Quiyang Zhang, Herman Poleo and Gang Yu.

Richard K. Cooper, Associate Professor, Department of Veterinary Science, and Terrence R. Tiersch, Professor, Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.

Development of a vaccine against *Flavobacterium columnare*

The two leading diseases affecting the catfish industry are the bacterial pathogens *Edwardsiella ictaluri* and *Flavobacterium columnare* (formerly *Flexibacter columnaris*). Treatment of these two pathogens has been difficult because only two antibiotics, Romet and oxytetracycline, have been approved by the Food and Drug Administration (FDA), and only one, Romet, can be effectively incorporated into feed. There is no vaccine commercially available for either bacterium, though at least one is undergoing tests for *E. ictaluri* (see page 17). To our knowledge, there has been no successful vaccination protection against *F. columnare*.

Traditional vaccines for livestock have consisted of a killed organism, or component of an organism, suspended in an adjuvant (a substance to stimulate the immune system), which is injected into an animal. Depending on the vaccine, a booster may be required later. This approach has been successful in domesticated livestock and in Atlantic salmon, where the cost of an individual animal is large enough to justify the expense of handling and injecting each animal.

Unfortunately, injectable vaccines are not an option for channel catfish because of the low cost of an individual animal. The only economically feasible vaccines for channel catfish are immersion and oral vaccines. An immersion vaccine is one in which the fish is completely immersed in a high concentration of the vaccine for a short time, usually 30 seconds to 2 minutes. The fish is vaccinated when the vaccine is ingested or crosses the gills. Oral vaccines are added to feed. Both methods depend on large amounts of vaccine being ingested and crossing the gut wall and entering the bloodstream, or entering the bloodstream directly by crossing the gills. Previous attempts in vaccinating channel catfish using killed *E. ictaluri* have resulted in low levels of protection.

Another approach is to use a live-attenuated vaccine, which is a live culture of a bacterium that has been altered by disrupting a gene so that it does not live long enough to reproduce in the host animal. Because it is live, the bacterium is able to enter the host on its own, but, being attenuated, it is no longer able to cause disease in the host. This approach allows sufficient numbers of the organism to enter the animal and be seen by the immune system.

Our goal is to develop a live-attenuated vaccine of *F. columnare* by genetically altering the chromosome (DNA) of the bacterium. Despite the economic impact of this organism in the catfish industry, surprisingly little is known about *F. columnare*. Though still in the early stages of the project, we are making significant progress in working with the bacterium. By identifying segments

of DNA (genes) responsible for how the bacterium can survive and cause disease in the host, we can gain a better understanding of the disease process. Genes required for the disease process can be interrupted (their function disrupted, possibly resulting in attenuation) using techniques developed for other bacteria. By placing *F. columnare* containing an interrupted gene back into the catfish, we can determine whether or not a particular gene is involved in the disease process and if the bacterium is attenuated. An attenuated bacterium is a good candidate for development as a vaccine.

The goal is to have the bacterium invade the fish and survive several days without causing disease or harm to the fish. The longer the attenuated bacterium survives, the longer the immune system has to establish protective mechanisms against the bacterium. The end goal is to have the fish develop a long lasting immunity against pathogenic (disease causing) *F. columnare*.

Development of a vaccine to *F. columnare* will provide fish farmers in Louisiana with another management tool that, if used correctly, will help lower the cost associated with producing fish. With the difficulty and expense in obtaining FDA approval on new



Photo by John Wozniak

In doing research on fish diseases, the researchers must screen the blood for antibodies. Two leading diseases affecting the catfish industry are the bacterial pathogens *Edwardsiella ictaluri* and *Flavobacterium columnare*.

antibiotics for food fish, the use of live-attenuated vaccines is an attractive alternative to increasing production and lowering production costs. ■

Acknowledgments

This work has been made possible with assistance from Brandye Smith, Jan Lousteau and Eric Amy.

Richard K. Cooper, Associate Professor, and Jacqueline McManus, Research Associate, Department of Veterinary Science, LSU Agricultural Center, Baton Rouge, La..

Benefits of induced sterility in prawn production

Although much genetic research involves developing ways to improve reproduction, it is just as important to develop methods to prevent reproduction. One reason is the potential for enhanced growth. Animals such as fishes invest substantial energy into reproduction that could be diverted to enhanced meat yield if they were reproductively sterile.

Sterility can be induced several ways, but most have not received much study in fish and shellfish. One method involves gamma radiation, a technique we are evaluating in the Malaysian prawn. The goal is to use sterility to improve yields and reduce size variation of cultured prawns.

Culture of freshwater prawns as a supplemental crop is rapidly gaining interest in the catfish-growing areas of the South. Barriers to profitable prawn culture include growth suppression and variation caused by social pecking orders. These pecking orders are based largely on sex, with a few large males dominating most of the population.

Studies in 1997 and 1998 indicated that it is possible to produce sterile males by gamma irradiation of juvenile prawns. Males irradiated at low doses had significantly lower numbers of sperm than did non-irradiated males. Males exposed to higher doses did not have sperm present in the testes at all. In females, the number of gravid and mated individuals decreased with higher dosages, and the number of virgin individuals increased with higher doses.

More research is needed to refine variables such as dose

levels and exposure periods, but in these initial studies, it appeared that although irradiation interfered with reproduction, it did not reduce survival. Because of small sample sizes, analysis of production traits such as weight and proportion of tail meat was not possible and is being pursued.

Unlike other techniques used to reduce obstacles to growth, irradiation can be done easily and cheaply. Commercially appropriate numbers of larvae could be irradiated in batches, adding a small cost to existing hatcheries. These larvae would represent improved seedstock that could be sold at a higher price. Their sterility would guarantee that producers would return to obtain new seedstock, thus protecting the investment.

If sterility can be induced reliably, another benefit will be to reduce concern about prawn culture in states, such as Louisiana, that strictly control introduction of non-native species and genetically modified animals. Irradiation of prawns could serve as a model for applying reproductive sterility in other aquaculture species such as fish or oysters. ■

Acknowledgments

Personnel involved in sterility research include Ingrid Ardjosoediro, Mark Bates, John Buchanan, Carmen Paniagua-Chavez, Greg Roppolo, William Wayman and Gang Yu.

Terrence R. Tiersch, Professor, and Nyanti Lee, Doctoral Candidate, both at the Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.

Vectors for transforming catfish

When a gene from one species of plant or animal is inserted into the genome of another species, the resulting plant or animal is then transgenic. Methods for transferring genes have one thing in common: the plasmid vectors used for gene delivery rely on random integration (insertion) of the transgene into the recipient chromosome. The result is usually an integration rate of 3 percent to 10 percent. With fish, it is usually 3 percent or less.

As we began to use transgenic techniques to investigate the possibility of enhancing disease resistance in channel catfish, it became apparent that a better method for obtaining stable integration was needed. Our goal has been to develop channel catfish resistant to disease by delivering to the catfish genome, as efficiently as possible, a gene encoding a lytic peptide. To circumvent the integration problems with previous transgenes, we designed a DNA vector (plasmid) that would force the incorporation of the desired gene into the targeted host genome.

Using a piece of mobile DNA called a transposon, we engineered a plasmid containing the lytic peptide within a transposon. A transposon can be cut or removed from the vector containing it (in this case a plasmid) by an enzyme called a transposase. By controlling the transposase with a promoter (on/off switch), we can regulate when the transposase removes the transposon from the plasmid and inserts it into another segment of DNA, that is, a catfish chromosome.

Once the transposon has been removed from the plasmid and inserted into the chromosome, the remaining plasmid DNA (containing the transposase gene) is destroyed. This removes the source of the transposase and eliminates the chance of the transposon being mobile in the future. The result is an efficient delivery system that creates stable DNA insertions with traits to be passed to the offspring. Work in our laboratory has resulted in more than 50 percent transformation efficiencies in channel catfish, and the transgene has been stable in these fish for more than three years.

Similar results have been obtained in koi carp. To demonstrate the ability of the gene to be passed from parent to offspring, we injected male koi carp in the region of the testes. Two weeks after injection, sperm were harvested and used to fertilize eggs. The offspring were assayed four times during a year for the presence of the transgene. Sixty-six percent of the offspring were positive after one year, and sperm from three of the five males remained positive for the transgene after one year.

This vector is a useful tool for delivering a desired gene to the genome of a wide array of animals and possibly plants. In addition to its value in producing transgenic fish, the vector has potential for being used in gene therapy applications to generate large numbers of cells with a transgene stably incorporated into their genome. ■

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New Disease Problems Continue to Arise in Aquaculture Industry

Ronald L. Thune and John P. Hawke

In much of aquaculture, animals are in high density production systems. This can result in stress from crowding and sub-optimal water quality conditions and provide for easy transmission of disease.

In response to anticipated disease problems, the Louisiana Aquatic Animal Disease Diagnostic Laboratory (LAADDL) was established in the LSU School of Veterinary Medicine in 1982. Initially, the laboratory assisted the few catfish and bait-minnow producers. However, as catfish farming expanded and technologies developed for the culture of additional species, the laboratory expanded its duties. Laboratory staff work cooperatively with several state agencies involved in protecting our aquatic resources. The LAADDL staff work with the Louisiana Department of Wildlife and Fisheries to diagnose disease problems in natural bodies of water and in the state's fish hatchery system. In addition, the lab helps the department's enforcement division to develop evidence to support the arrest and conviction of individuals involved in the illegal taking and selling of wild fish. The staff is also a part of the LSU School of Veterinary Medicine Fish Kill Emergency Response Team, which helps the Louisiana Department of Agriculture and Forestry and the Louisiana Department of Environmental Quality investigate fish kills.

Most case submissions to the LAADDL, however, have been from commercial aquaculture, involving catfish, hybrid striped bass, soft-shell crawfish and tilapia. Several of the most significant problems are highlighted below.

Catfish

A primary problem in catfish farming is a bacterial disease specific for channel catfish called enteric septicemia of catfish, or ESC. Control involves giving antibiotic treated feeds to the fish. Only two antibiotics, Romet and oxytetracycline, have been approved by the Food and Drug Administration (FDA). Limited treatment options led to overuse of the individual antibiotics and the development of ESC bacteria

resistant to one or both drugs. Alternate treatment options developed, including cessation of feeding in affected ponds to prevent oral exposure of the fish to the bacterium in the water. It was apparent, however, that the preferred option was prevention of disease by vaccination, so we initiated research to develop an effective vaccine. This proved more difficult than anticipated, but has led to the development of an effective product that is being field tested (see page 19).

In the mid-1980s, aquatic diagnostic labs in Arkansas and Mississippi reported a new disease problem that farmers called "Hamburger Gill Disease" (HGD) because of the massive destruction that occurred in the gill filaments. In April 1985, the first case in Louisiana was diagnosed from a newly constructed pond. More than 90 percent of newly stocked 4-inch to 6-inch catfish died within 48 hours to 72 hours of stocking, and similar scenarios were repeated more than 60 times in the next 36 months as new ponds were constructed all over the state.

At the time, HGD was an emerging disease problem with an unknown cause. Because of the high mortality rates and the lack of an available treatment, LAADDL staff recommended rapid harvest if the fish were of marketable size, followed by a complete disinfection of ponds with quick lime. Six years later a protozoan parasite was confirmed as the causative agent. Soon after that, a small aquatic worm was implicated as an intermediate host for the parasite, releasing a small spore into the water that infected the fish. The catfish was an accidental host for the parasite, however, because the parasite was never able to complete its life cycle and produce a stage that could reinfect the worm. At present, with still no treatment available, efforts continue to find a control for the worm host and to identify the true final host. Elimination of the worm host or the final host are the only options for controlling HGD-associated deaths.

Crawfish

Farm-raised crawfish seldom experience disease outbreaks. While

growing rapidly, they constantly molt the exoskeleton and, in the process, shed any external parasites. Also, crawfish production is not nearly as intensive as the other aquaculture systems in the state, so the stress/crowding-associated diseases are uncommon. But, in the late 1980s, two developments in the industry led to intensification.

First, attempts to expand crawfish markets to non-traditional markets in other states found that consumers were reluctant to eat crawfish with the dark vein (actually intestine). This led to the development of high density purging systems in which crawfish were held for two to three days without feed to allow the intestinal tract to become void. The resulting product looked cleaner and was more acceptable.

Second, the softshell crawfish phenomenon swept through Louisiana. Using technology developed at the Louisiana Agricultural Experiment Station, producers held crawfish at high density in tray systems, selected crawfish as they shed their exoskeletons and sold them to the restaurant trade for more than \$7 per pound.

Unfortunately, as in other situations, harvest and movement to the intensive, crowded conditions stressed the crawfish. In 1985, unexplained mortalities began occurring in an experimental purging system at the Ben Hur Aquaculture Facility, now the Aquaculture Research Station. A non-O1 strain of *Vibrio cholerae* was the cause. Over the next several years, *V. cholerae* was isolated from a number of purging and softshell operations that were experiencing significant die-offs. Recommendations to reduce harvesting stress by running traps more frequently and to reduce transport stress by keeping the animals cool and moist helped alleviate the problem somewhat. Eventually the

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softshell industry succumbed to economic problems associated with overproduction, but *V. cholerae* is occasionally diagnosed in the remaining facilities.

Tilapia

In August 1992, the Department of Wildlife and Fisheries issued the first permit for a tilapia farm in Louisiana. It was very restrictive and limited to indoor, recirculating, intensive systems. The success of the initial farm attracted additional producers, and Louisiana now has five. As with other species, the appearance of a serious disease was inevitable and, in 1994, we began to receive tilapia specimens from all over the United States. A serious bacterial pathogen, *Streptococcus iniae*, was isolated from the diseased specimens. Fortunately, the disease was not reported from Louisiana farms. Because of the

seriousness of the *S. iniae* problem in other regions, LAADDL staff recommended that Louisiana producers not import any tilapia, and, if importation was necessary, to apply strict quarantine procedures. This recommendation was followed, and as of this writing *S. iniae* has not been reported from Louisiana tilapia farms. It remains a significant problem elsewhere.

Hybrid Striped Bass

In the late 1980s, intensive culture of hybrid striped bass increased dramatically in Louisiana and surrounding states. This was reflected in submissions of hybrid striped bass specimens, which increased from 18 cases in 1988 to 220 in 1991. Parasitic and bacterial infections were common in the early development of the industry, all of which could be controlled with treatment.

In 1990, hybrid striped bass culture spread to the coastal marshes where fish were raised in cages and raceways. That December, a new bacterium, known as *Photobacterium damsela* subsp. *piscicida*, was isolated on one of the coastal farms. Photobacteriosis proved difficult to control because the fish stop eating, which precludes treatment with antibiotics in feed.

Over the next several years, photobacteriosis caused massive mortalities and was at least partially responsible for closing several fledgling hybrid striped bass farms on the coast, where it remains a major constraint to the development of fish culture. Recent research has led to the development of a live attenuated vaccine for photobacteriosis that has promise for preventing the problem worldwide (see page 15). ■

Photo by Greg Lutz



In Louisiana, tilapia can be grown only indoors since they are not a species native to the state. This regulation may be lifted pending further research at the Aquaculture Research Station.

Preventing enteric septicemia of catfish and photobacteriosis in hybrid striped bass

Two serious problems affecting Louisiana aquaculture are the bacterial pathogens that cause enteric septicemia of catfish (ESC) and photobacteriosis in hybrid striped bass. ESC causes the loss of millions of dollars annually in the catfish industry. Photobacteriosis restricts the development of hybrid striped bass culture in coastal Louisiana and causes financial losses in the Mediterranean region and Japan.

The only tools for controlling these diseases are antibiotics used after clinical signs appear. Because the antibiotics are delivered in feed, and because sick fish often cease feeding, the "treatment" actually prevents disease in the fish not yet infected. Effective vaccines, on the other hand, act to prevent disease by stimulating the natural immune system of the fish. The immune system becomes "primed" in vaccinated fish, increasing the ability of the immune system to recognize and kill the bacterium before establishment of disease.

Researchers previously vaccinated against ESC and photobacteriosis by immersing fish in a bacterial cell suspension that was killed by the addition of formalin. Vaccination by injection, the method of choice for human and animal medicine, is not economically feasible for catfish or hybrid striped bass because of the small size at vaccination, the large numbers of fish and the relatively low value per individual fish. Protection from disease using these killed vaccines, however, was very weak, partly because of the poor uptake of killed vaccines by immersion. Other studies indicated that both bacteria can survive and grow within living cells in the fish. By "hiding" in cells, the bacteria are able to avoid the parts of the immune system that are most strongly activated by killed vaccines, resulting in a weak protective response.

To induce a protective immune response, it was apparent that a larger dose of vaccine needed to be delivered, and that it needed to be delivered in a way that more closely mimicked the natural, intracellular location of the infection. Because both of the bacteria that cause ESC and photobacteriosis are capable of rapid invasion of the fish from the water, live vaccine strains that retain this invasive capability will "inject" large doses of vaccine. If the live vaccine also retains its intracellular residence, it will stimulate the parts of the immune system that are important in controlling bacteria that can hide in host cells. The combination should result in a safe, effective vaccine that will protect fish from disease.

With support from the Louisiana Catfish Promotion and Research Board, Alpharma Inc. in Seattle, the Louisiana Education Quality Support Fund, the National Sea Grant Program and the U.S. Department of Agriculture (USDA), we have successfully

developed and are evaluating live vaccine strains that prevent ESC and photobacteriosis. Using molecular genetic techniques, we deleted from each bacterium a gene required for growth. Its deletion does not affect the bacteria's ability to invade fish and establish an initial infection within host cells. The bacteria can infect the fish and distribute themselves in the cells, but their growth is limited because of the deleted gene. As a result, the fish clear the bacteria from their bodies within 48 hours to 72 hours. Because the gene deletion was carefully constructed and involves



Photo by Greg Lutz

In the late 1980s, intensive culture of hybrid striped bass increased dramatically in Louisiana and surrounding states. Photobacteriosis is a disease that threatens this industry.

a large portion of the gene, the vaccine strain cannot revert to the wild, disease-causing strain.

During the abortive "infection" with the live vaccine strain, the fish immune system interacts with the bacteria as it would in the early stages of a natural infection, stimulating the part of the immune system required to protect against intracellular bacteria. Essentially, we have created a version of the bacteria that "injects" itself, but does not cause disease. In our experiments, we have demonstrated that vaccination with the vaccine strains results in significant protection from disease after exposure to the wild, virulent bacteria. The ESC vaccine is approved for field testing by the USDA, and experiments are under way to generate data to support a full vaccine license application to the USDA. Preliminary data for the photobacteriosis vaccine are almost completed, and an application for field testing will be submitted to the USDA in 1999. ■

Ronald L. Thune, Professor, Departments of Veterinary Science and Veterinary Microbiology and Parasitology, and John P. Hawke, Assistant Professor, Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Baton Rouge, La.



Ornamentalspecies

Stripedbass

Cocahoeminnows

Potential for Increased Diversification in Louisiana's Aquaculture Industry

C. Greg Lutz

Although Louisiana already has a diverse aquaculture industry, many more species could be grown here or grown on a larger scale. Many prospects present specific problems that could complicate commercial development, but most of these constraints relate to marketing, financing or regulatory considerations, not technical issues. This article addresses some of these potential species in the order of most promising to least promising.

Hybrid striped bass

Perhaps the most promising finfish candidate for future commercial development in Louisiana is the hybrid striped bass. In the past decade, successful hybrid striped bass culture has been demonstrated in northeast, south-central and coastal areas of the state. Although Louisiana has more than 18,000 acres of commercial catfish ponds, only 15,500 are in production. Many are suitable for commercial production of hybrid striped bass.

Existing markets for hybrid stripers are mostly on the East Coast. These outlets could probably absorb added production without significantly reducing profitability, but substantial increases in production will require distribution into new markets. Fortunately, this fish could potentially fill many supply gaps resulting from reduced availability of traditional species along the Gulf and Atlantic coasts. LSU Agricultural Center research on hybrid striped bass focuses on immunization against major diseases, potential for development of domesticated hybrid-based strains, production strategies to spread harvests throughout the year and other topics.

Cocahoe minnows

The popularity of cocahoes as bait for recreational marine species has remained high. Commercial suppliers depend on wild-caught minnows, and they have failed to keep pace with demand. Techniques for culturing cocahoes were first investigated in Alabama in the 1970s. Later work in Texas outlined recommended approaches to cocahoe production, based on research and commercial results there and elsewhere. Net, after-tax revenues of

\$2,990 per water-acre were projected, and potential markets for farm-raised minnows were identified along most of the northern Gulf of Mexico.

Aquatic plants

Louisiana has the climate, soil and water resources to propagate commercial algae, ornamental plants for aquariums and water gardens, and marsh and wetland vegetation for restoration or mitigation projects. Markets are expanding rapidly, and these aquatic crops could be quite profitable.

Ornamental species

Increasing popularity of water gardens throughout the southeastern United States has created demand for koi carp and fancy goldfish. One of the country's premier koi farms operates in Pointe Coupee Parish. Potential also exists for commercial production of many freshwater aquarium species in Plaquemines Parish, south of Pointe a la Hache, in small ponds similar to those used by producers in the Tampa Bay region.

C. Greg Lutz, Associate Specialist (Aquaculture), Louisiana Cooperative Extension Service, LSU Agricultural Center, Baton Rouge, La.



Freshwaterprawn

Bream

Tilapia

Redfish

Illustration by Elma Sue McCallum

Tilapia

Tilapia is the common name for a group of tropical, perch-like species native to Africa and the Middle East but introduced to tropical regions worldwide. Tilapia production has increased dramatically in North America in response to Asian and Hispanic demand for live fish in metropolitan areas. Outdoor production of tilapia is legal in Arkansas, Mississippi and Texas, but Louisiana's Department of Wildlife and Fisheries requires these fish to be kept in indoor recirculating systems to prevent their escaping to local waters. This increases production costs considerably, but reduces seasonality and depressed prices associated with the harvest of these tropical fish from ponds before the first cold front each winter.

Greenhouse-based systems developed by Louisiana growers and Cooperative Extension Service personnel achieve lower production costs than most other U.S. operations. Unfortunately, tilapia farms have been constructed throughout the United States in recent years, and prices in live markets have plummeted as a result. For the past eight to 10 months, most tilapia going to live markets have been sold at significantly less than production cost because late arrivals to the industry have been faced with the need to service start-up debt.

In Central America, northern South America and the Caribbean basin, commercial production of tilapia has expanded rapidly to target North American fillet markets. If U.S. tilapia

producers are to avoid substantial losses and compete in fillet markets, production costs must be further reduced through adoption of improved stocks. Research in this area is a cooperative effort of the LSU Ag Center and commercial producers. Results from analyses of Louisiana greenhouse systems suggest that using faster growing strains in conjunction with improved filtration systems could potentially lower costs to produce and deliver fresh fillets to levels comparable with estimates for imported product.

Softshell crawfish

The seasonality that has plagued the softshell crawfish industry might be reduced by using an alternative, complementary species of crawfish. *Orconectes lancifer* is a shrimp-like crawfish native to many parts of Louisiana. It tolerates high temperatures and low oxygen, spawns in late winter and grows throughout the summer and fall when normal supplies of shedding crawfish are unavailable.

Redfish

A moratorium on commercial redfish, or red drum, harvests from the Gulf in the 1980s led researchers and entrepreneurs to devote considerable resources to the development of aquaculture techniques for this species. Budgets developed for red drum production in the late 1980s and early 1990s were based largely on preliminary research and optimistic assumptions. As experimental data from public institutions and private ventures became available, however,

original estimates of profitability often proved overly inflated. Research and commercial efforts devoted to red drum culture declined steadily through the 1990s as temperature, disease and cash flow risks were increasingly perceived to outweigh the potential for profits.

Few areas of research stand out as avenues to expand commercial production of red drum. One approach that may hold some promise is recirculating tank production. In recent years, equipment options and management strategies available to producers who use indoor recirculating methods have improved dramatically. Red drum adapt fairly well to tank culture, as evidenced by success at various research facilities.

Freshwater prawns

Production of the tropical Malaysian prawn is biologically feasible in many southeastern states, including Louisiana. As with tilapia, the need to complete pond harvests each fall before cold weather results in marketing constraints. Additional problems relate to limited availability and high cost of juveniles for stocking in late spring. Ag Center research with this species focuses on production of all-male or sterile stocks in an effort to improve yields and reduce size variability at harvest.

Eels

Eels have long been a valuable aquaculture crop in Europe and the Far East. Captive spawning is not practical, but production technologies are well documented, and wild-caught elvers are

occasionally available along the Gulf Coast. Although global competition might limit export opportunities for this species, regional and live markets might absorb considerable production.

Hybrid bream

This fish is primarily a live market item, but there is limited demand for dressed fish in New York as well as Chicago and other Midwestern markets. The degree to which prices will drop with any increase in supply remains to be seen.

Shrimp

Little or no potential exists for economically viable marine shrimp farming in Louisiana, but sophisticated hatchery operations producing high-quality, pathogen-free post-larvae for export to shrimp-producing countries in Central and South America could be profitable.

Bullfrogs

Several major problems with frog culture remain unsolved. When combined with comparatively high land and labor costs and limited growing seasons, these constraints have prevented the development of commercial frog culture in the United States. Up to 1.15 pounds of live food is required to produce a

single 0.4- pound bullfrog destined for the frog leg market, and bullfrogs will not voluntarily consume artificial diets that lack the appropriate combination of movement, texture and flavor. Approaches to commercial frog culture in tropical regions can be adapted to allow laboratory-scale production of small frogs for research and teaching purposes, but high costs rule out commercial frog leg production in the Southeast.

Offshore cage production

The technology exists to build offshore fish cages that can easily withstand conditions in the Gulf, and many offshore platforms are already in place to support production operations. No outstanding high-value candidate species presents itself for conditions in the Gulf, but future domestication efforts may result in availability of fingerlings of one or several species from the snapper-grouper complex or possibly some type of flatfish. Successful net-pen siting depends on finding currents that freshen the water within the net-pen on a continual basis and disperse feces and uneaten feed across a wide swath of water bottom to avoid buildup of anoxic sediments. Criticisms of net-pen farming in other parts of the world include the assertion that captive fish serve as reservoirs for diseases and parasites.

Offshore production will probably require on-shore facilities for hatchery and nursery operations. These might have considerable siting problems in the coastal zone.

On the horizon

A number of species are being considered or actually evaluated for commercial potential in the Southeast. When evaluating the ability of Louisiana producers to compete with imported products, key considerations for any species involve the relative costs of production, processing and distribution. In spite of high transportation costs, tilapia producers in Central and South America can put a fillet on the market in Louisiana for a much lower cost than local producers can. In the case of live fish or aquatic plants, however, high transportation and distribution costs far outweigh production advantages of foreign producers, effectively preventing competition from imports. As technology evolves and demand for seafood increases, it is almost a certainty that new species will become available to Louisiana producers. Technology and expertise, however, cannot foster aquaculture development in Louisiana without a parallel emphasis on regulatory, financial and marketing support for this industry. ■

PhotobyGregLutz



The technology exists for marsh mariculture and offshore fish cages that can easily withstand conditions in the Gulf of Mexico, and many offshore platforms are already in place to support production operations.

More A'peeling Mudbugs

Purging Crawfish Holds Promise for More Profits

W. Ray McClain

The preferred way to cook crawfish is to boil the whole animal in seasoned water and then serve it so the consumer extracts the abdominal muscle, or tail meat, by hand. When the exoskeleton is peeled from the abdomen, the intestine is often exposed and can be unappealing. The full, dark-colored intestine of unpurged crawfish contrasts conspicuously with the light-colored meat. It can be particularly offensive if the intestinal wall is ruptured during peeling, contaminating the meat with gritty fecal material.

For a more attractive product, crawfish are sometimes placed in depuration or "purging" systems for one or two days. Crawfish are confined in water or high humidity environments and food is withheld. This process cleans the exoskeleton of mud and debris and eliminates or greatly reduces digesta in the intestine. The intestine of a fully purged crawfish is smaller, translucent and much less conspicuous. Because purging removes ingesta from the stomach and cleans the gill chamber of grit and mud, the water used to boil crawfish does not become contaminated.

Placing crawfish in salted water for several minutes before cooking is sometimes improperly referred to as purging, but this process does little to remove contents of the intestine and is little more than a cursory wash. The salt has little effect.

Some people who have eaten crawfish for many years are accustomed

to non-purged crawfish and do not find them objectionable. Nonetheless, most would prefer a purged product if the additional costs were minor. Purging has contributed to repeat sales and loyalty to certain suppliers and has helped foster new markets in non-traditional locales.

Because costs of purged crawfish are 15 percent to 25 percent higher, the current market for purged crawfish is

involves totally immersing the crawfish. The spray system involves exposing the crawfish to water spray or mist. Both types were evaluated for their effect on crawfish survival and evacuation rate of intestinal contents. The findings showed that the systems were similar.

Crawfish mortality during purging averaged 8 percent after 24 hours and 12 percent for 48 hours and was similar to

previous findings. Of the total amount of intestinal contents excreted during 48 hours of purging, 70 percent, on average, was excreted within the first 12 hours. Because purge-related mortality increases with time, the shortest acceptable purge duration will be the most cost effective. A 12-hour (or overnight) purge is sufficient for cleaning the exterior of crawfish and for the evacuation of most of the intestinal contents. A 12-hour duration will incur the least mortality

and use less energy. Therefore, approximately 12 hours is likely to become the recommended purge duration.

Research is evaluating ways to increase the efficacy of purging. Preliminary findings suggest that segregating crawfish by size and decreasing the loading rates during purging lessens mortality. ■

W. Ray McClain, Professor, Rice Research Station, Crowley, La.



This is an immersion purging system at the Rice Research Station used in research. John Sonnier, research assistant, pours the purged crawfish onto a tray for sorting.

relatively small. The higher cost of purged crawfish comes from the capital expenditures and operating expenses needed for purging, the extra labor required and crawfish death associated with the purging process. Research has been conducted at the LSU Agricultural Center's Aquaculture and Rice Research stations to better characterize the purging process and to develop protocols for reducing cost.

Two basic types of purging systems have been used in the industry. Immersion, which is the most common,

Quest Continues for Rice Variety Tailored to Crawfish Production

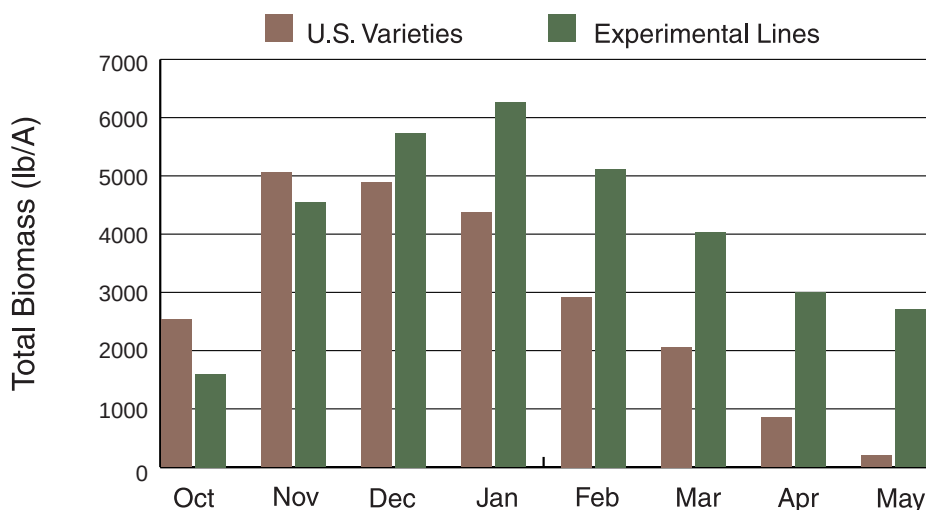
W. Ray McClain and Steven D. Linscombe

Crawfish aquaculture in Louisiana depends solely on a forage-based food system for supplying nutrients to the growing animals. Because of available plant residue following grain harvests and because rice exhibits good regrowth characteristics, crawfish production often follows the rice harvest as a common crop rotation practice. Not all use of rice forage in crawfish production occurs as a result of rice-crawfish multi-cropping practices, however. A substantial portion of the crawfish production occurs in mono-cropping systems, where crawfish is the only crop harvested. Rice is often the preferred forage crop in those systems and is planted strictly for its desirable forage characteristics and not grain production. Although rice has proved to be, on average, the best forage resource, it is often depleted prematurely in ponds with large numbers of crawfish. This causes food shortages for the remainder of the production season, resulting in stunted crawfish. Since crawfish are valued according to harvest size, this causes an economic disadvantage.

Currently, only common domestic rice varieties, bred for grain production, are available for mono-cropping systems. The forage characteristics of today's domestic rice varieties, however, are less desirable under flooded crawfish culture conditions than those of the past. Rice breeders have developed varieties for grain production that have higher grain-to-forage ratios, are shorter in plant height and mature earlier. These characteristics render them less desirable as crawfish forage sources. As the gap widens between the desirable traits of the older varieties and the newer grain-producing varieties, the need has grown for a crawfish-specific rice variety with more desirable forage traits.

Research has been under way at the Rice Research Station since 1991 to evaluate rice genotypes for characteris-

Figure 1. Comparison of average total forage biomass (three-year average) by month for the U.S. varieties Mars and Cypress and the three experimental lines.



tics desirable in crawfish culture. More than 16,000 genetic lines from the U.S. Department of Agriculture Rice World Collection, as well as current and older domestic varieties, have been screened. Desirable traits, such as extensive forage

production, cold tolerance, long maturity cycle, high resistance to lodging, slow depletion rates, disease resistance and propensity for forage regrowth in early spring, were some of the criteria for selection. Large differences in forage



Figure 2. When planted in early August, the domestic rice variety Cypress (far center) reached maturity in November and began its decline much sooner than the experimental genotypes (foreground and background) that did not reach maturity.

W. Ray McClain, Professor, and Steven D. Linscombe, Professor, Rice Research Station, Crowley, La.

GENOME MAPPING of aquaculture species

attributes were observed, and three outstanding candidates—short grain types originating in Taiwan, Fiji and China—were selected for further trials.

In preliminary field trials conducted over three years in small, experimental crawfish ponds, the improved exotic lines consistently outperformed the varieties Mars, an industry standard for years, and Cypress, the most currently used variety. There were few differences among the experimental lines, but those genotypes produced greater amounts of forage than did the domestic varieties, and the forage availability during the later months of the crawfish production season was much greater (Figure 1).

Although on average, total crawfish production (averaging more than 1,100 pounds per acre) with the experimental lines was no greater than with the domestic varieties, a greater proportion of large, high-value crawfish were produced in ponds planted with the experimental genotypes. The proportion of crawfish grading into the largest size category was increased by 14 percent to 20 percent with the experimental lines. This most likely occurred because of the increased forage residue late in the production season, often a time of severe food shortages.

Moreover, in preliminary yield tests conducted under typical rice-growing conditions, all three experimental lines averaged grain yields comparable to current domestic varieties. Milling yields of two of the lines were satisfactory, too. Acceptable grain yields would be essential to seed growers for the economic propagation of seed. Good milling yields might encourage production of rice for niche grain markets, providing greater incentives and markets for the seed producers.

Further evaluations of the experimental lines and crawfish production will be conducted, and improvements will be sought through additional purification during seed increases. Pending acceptable results of those tests, one or more of the experimental lines will be evaluated for immediate release as the first crawfish-specific rice variety or incorporated into the Rice Station's rice breeding program for further development. ■

Geneticists develop maps of DNA molecules to aid in understanding inheritance patterns. One kind of map, called a genetic linkage map, describes inheritance of observable traits, such as color or shape, and usually involves breeding studies to compare parents and offspring. Physical genome maps describe the actual geography of chromosomes, the DNA-bearing structures within the control center (nucleus) of a cell. These maps do not require breeding studies. Physical maps are derived mainly from chemical measurements made on the DNA molecules of an organism, referred to collectively as the "genome." These maps can identify the genes that carry the blueprints for the proteins necessary for life or stretches of genetic material with no known function. In the Human Genome Project, a massive research effort to characterize the complete DNA sequence of human beings, both of these map types are considered equally important and are being developed in parallel.

Genetic linkage studies of economically important fishes, such as salmon and trout, are fairly well established and have been started recently for channel catfish. But little work has been done in aquatic species to address physical genome mapping. Until recently, all that was known about channel catfish was that they have 29 pairs of chromosomes. The fact that humans have 23 pairs has been known for decades. Despite the economic importance of channel catfish, little information exists about genetic markers for production traits such as improved growth and disease resistance. This is the situation for all of the aquaculture species of Louisiana.

A chromosome map provides landmarks for gene location. Techniques are well developed for mammalian chromosomes to produce multiple high-resolution markers called bands. These techniques have yielded little success when applied to fish species, however. Fish chromosomes are more difficult to study than human chromosomes for several reasons including their large numbers (sometimes more than 100), small size (a third of the size of human chromosomes) and uniformity. There are many of them, and they all look alike. A long-term team goal is to prepare detailed chromosome maps (called banded karyotypes) for the cultured fish and shellfish of Louisiana. Based on this information, we will identify the locations of genes of economic importance, such as for disease resistance and growth rate, and develop DNA markers to use in genetic improvement including hybridization, selective breeding and gene transfer studies.

Because of genetic similarity among organisms, physical mapping of catfish or oyster genes can be performed with information obtained from mammalian species, including humans. This offers significant benefits from comparative studies and demonstrates a direct application of well-funded human medical research to the study of aquaculture species, which receives much less funding and effort. We have developed techniques to identify the location of individual genes on chromosomes of fish and shellfish. Our laboratory was the first to map genes of catfish and oyster (or any aquatic species) by a technique known as *in situ* polymerase chain reaction. This technique identifies the gene location by making copies of the DNA, which can be labeled and identified using a microscope. ■

Acknowledgments

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Better Crawfish Aquaculture Through Research

W. Ray McClain and Robert P. Romaine

The domestic crawfish industry is the only large-scale, commercially viable crustacean aquaculture industry in North America. With more than 110,000 acres of crawfish ponds, Louisiana's 1,600 farmers produce 35 million to 50 million pounds annually worth \$25 million to \$35 million at the producer level. Another 800 commercial fishers harvest crawfish from natural wetlands, primarily the Atchafalaya River Basin, the largest overflow swamp in the United States.

Annual harvest from the Basin varies with water discharge that depends on precipitation patterns in the middle and upper Mississippi River Valley. Harvest may be 60 million pounds in high water years and almost nothing in low water years. These unpredictable harvests, combined with increased consumer demand, provided much of the impetus for the development of crawfish aquaculture. The beginning of crawfish aquaculture in Louisiana was somewhat humble with only about 40 acres in 1949, but the industry has grown from an incidental crop to a vital agronomic venture.

Crawfish aquaculture is managed either as a sole crop or as part of integrated farming enterprises in which rice is the principal crop. To use natural and economic resources efficiently, many rice producers double-crop crawfish in rice fields after the rice harvest. More than half the crawfish in Louisiana are now cultured in rice fields (Figure 1).

Crawfish evolved in seasonally flooded wetlands, and current pond production is based on the annual wet/dry cycles to which the crawfish have become adapted. Unlike the culture of many aquatic species that require hatcheries and formulated feeds, crawfish culture is based on self-sustaining populations that use a forage-based food web for acquiring nutrition. Crawfish retreat to burrows to survive the summer dry period and to reproduce (Figure 2). Growth of the offspring occurs in the shallow, forage-laden ponds during the fall, winter and spring, and market size animals are harvested

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Photo by Mark Claesgens

Figure 1. Research assistant John Sonnier is harvesting crawfish at the Rice Research Station. Louisiana has more than 110,000 acres of crawfish ponds.



Photo by W. Ray McClain

Figure 2. Crawfish retreat to reproduce and survive pond drawdown during the summer. Research has documented that most burrowing occurs in pond levees and not in the pond bottom.

with baited traps from about December through May.

Before 1987, there was no established pricing system based on graded and sized crawfish. Pricing was influenced largely by supply and demand with little regard to size, above a minimum acceptable level. The principal emphasis of management for the crawfish producer was to maximize total production of harvestable crawfish.

In 1987, a major export initiative of crawfish to Sweden prompted the crawfish industry to establish grading practices, which subsequently influenced the production and marketing of crawfish and influenced crawfish research efforts. The export market was lucrative and demanded only select crawfish of the largest size. To effectively segregate crawfish for this market, the industry devised grading processes. The establishment of grading in the industry allowed not only the segregation of crawfish for export but also for domestic markets. Grading by size became a standard industry practice. Crawfish are routinely graded into three size grades: large for the high-value export market, medium for the domestic live or restaurant markets and small for the processed tail meat market.

Historically, the most significant problems facing Louisiana crawfish producers have been low yields, harvest of small, sub-marketable crawfish, or low profitability resulting from inefficient harvesting techniques. Low yields result from reproductive failures and

deaths, usually juvenile crawfish. Production of small or stunted crawfish occurs mainly from overpopulation, often exacerbated by food shortages and sometimes poor water quality. Inefficient harvesting occurs when trapping intensity is insufficient or too great, or when the most efficient traps or baits are not used.

Since 1966, Louisiana Agricultural Experiment Station (LAES) researchers have been addressing the needs of Louisiana crawfish farmers. They have a strong track record of developing new technologies that have been adopted by the industry. Ag Center research has led to a better understanding of the factors that affect crawfish production, including identifying poor water quality (low dissolved oxygen) as the primary cause for massive mortalities of newly hatched crawfish. Water management strategies (such as delayed flooding and water circulation and flushing) were developed and are used by producers to manage for better water quality. Research determined that planted forage crops were the most effective for establishing a sustaining food system and found rice to be the most dependable forage.

Today, most crawfish farmers plant rice as the forage crop. Supplemental feeding of agricultural byproducts and formulated feeds was investigated and, although found to be somewhat effective, it was usually not economical. Harvesting research determined the optimum density of traps, optimal bait soak time and, probably most significant,

led to the development of formulated (pelleted) baits that replaced much of the fresh-frozen fish baits. Today, feed manufacturers sell \$4 million to \$5 million of formulated crawfish bait each year, nearly half of the total crawfish bait market.

Research during the 1990s has continued to address production needs of the crawfish industry, with special focus on producing larger crawfish. Small crawfish are least valuable, but the highest percentage of the cultivated crawfish crop traditionally falls into the smallest category.

Compounding the problem of small crawfish, beginning in 1991, Louisiana's crawfish industry was hurt by massive imports of crawfish tail meat from China at below fair market value. In 1996, 8.5 million pounds of imported crawfish tail meat (nearly 56 million pounds of live weight equivalent) displaced more than 90 percent of Louisiana's domestic crawfish meat market. Crawfish producers lost markets for small crawfish that were traditionally peeled and were subsequently deprived of a significant source of income. Although a tariff was imposed on most of the importers in 1997 and eased the situation, research emphasis has been directed at production of larger crawfish and improving production efficiency.

LAES researchers determined that overpopulation is the single most important factor affecting crawfish size at harvest. Renewed scientific investigations into supplemental feeding in the 1990s showed that harvest size and yields could be marginally increased by feeding, but feeding negatively affected catch by interfering with the effectiveness of the baited trap, and feeding alone could not negate the effect of overcrowding. Researchers learned that overcrowding was caused by many factors, including a high levee area to pond surface ratio that increases the burrowing area for crawfish. It was determined also that extensive levee renovation hurt crawfish production by destroying adult and newly hatched crawfish in the burrow. Producers, dependent on natural recruitment from broodstock contained in the pond, have little control over populations but now have a better understanding of crawfish population dynamics and that relationship to production. Means to control overpopulation and to augment low populations have been the focus of recent research efforts by LAES scientists; although promising results have

been attained, few recommendations have yet been developed.

Harvesting costs account for 50 percent to 70 percent of the cost of producing farm-raised crawfish, and findings from harvest research conducted by LAES scientists have translated into significant savings in bait and labor costs. Reductions in harvesting days and bait use with the more efficient pyramid trap (Figure 3) was achieved without reducing yield, but with significant savings in harvesting cost. Additionally, it was shown that with reductions in trapping intensity, larger crawfish could be harvested. Industry adoption of these harvesting practices is estimated to have saved the industry \$2 million to \$3 million annually since 1993. More recently, research has determined that trap harvesting becomes more efficient when bait type is alternated daily. This is a “no cost” management tool thought to work by maintaining a more distinct scent gradient, thus increasing the attractiveness of the bait.

The LSU Ag Center is committed to working on behalf of the Louisiana crawfish industry and will continue to address production concerns of the crawfish producers through applied



Photo by W. Ray McClain

Figure 3. The efficient pyramid trap has become the industry standard used to harvest crawfish from culture ponds. Much of the current harvesting strategy was developed by scientists with the LSU Agricultural Center.

research. Financial support of crawfish research has been provided from several sources including the Louisiana Crawfish Promotion and Research Board (through check-off funds), USDA

Special Grants Program, the Louisiana Board of Regents LEQSF program and the USDA's Southern Regional Aquaculture Center. ■



Photo by John Wozniak

Harvesting costs account for 50 percent to 70 percent of the cost of producing farm-raised crawfish, and findings from harvest research conducted by LAES scientists have translated into significant savings in bait and labor costs.

Making use of Louisiana's undersized crawfish

Numerous studies have been done on value-added new product development from byproducts and waste of seafood processing operations or from underused fish species, but the only research on use of minced meat recovered from undersized crawfish is at the LSU Agricultural Center. Understanding the properties of crawfish minced meat for use in subsequent new product development is critical. The minced meat has 77.5 percent moisture, 1.2 percent fat and 14.5 percent protein. The shelf life can be up to 6 months if vacuum-packaged and stored at minus 18.4 degrees F. The color remains similar to that of the fresh tail mince, which is encouraging since freshness of crawfish is the most important factor for commercialization, according to the market analysis of new crawfish tail mince products from undersized crawfish.

Our preliminary work identified potential products, including crawfish dip or butter, seafood sauces, patties, nuggets or sausage. Markets identified include seafood restaurants in Louisiana, Mississippi and the Texas Gulf Coast. More than 70 percent of restaurant managers and chefs in these markets responding to a mail survey indicated willingness to buy a base or stuffing product prepared from minced crawfish. Freshness, followed by price and flavor, are important attributes. For the minced meat crawfish base or stuffing to be successful, they must be a fresh product with a hearty crawfish flavor, and they must be priced at 30 percent to 70 percent of the cost of the fresh tail meat.

Crawfish nugget

The crawfish nugget is an example of a product prepared from minced meat recovered from underused undersized crawfish and was initially developed in 1997 by researchers in the Department of Food Science and Department of Agricultural Economics and Agribusiness. The product development involves several steps including a preliminary market study, product formulation, process development, quality assessment, consumer acceptance evaluation and market tests. The product was formulated to have a hot-spicy taste and contain more than 50 percent crawfish minced meat. The fried nuggets, depending on formulation, have 12.7 percent to 18.4 percent protein, 16.4 percent to 20.6 percent fat, and 36.6 percent to 45.4 percent moisture.

Eleven nugget products were formulated and used in consumer taste tests in 1998. A total of 177 consumers participated in this study. About 73 percent indicated that "taste" is the most critical sensory attribute. The "hot-spicy" and "salty" are the most preferred tastes. Consumers also were concerned about the price (60.5 percent) and the size and shape of the product (22.6 percent). Consumers were asked to evaluate appearance, color, flavor, surface texture, overall



Photo by John Wozniak

Witoon Prinyawiwatkul conducts consumer taste panels in his laboratory facilities. There are two different products on his plate, a crawfish nugget and a catfish "finger."

texture and overall liking of the products. They also classified each crawfish nugget product as "acceptable" and "unacceptable" and indicated whether they would buy or not buy the products.

Of the 11 formulations, two show market potential. The acceptability scores for appearance, color, flavor, surface texture, overall texture and overall liking were higher than 7 (moderately like) on a 9-point scale. More than 90 percent of the participating consumers indicated that these two products were acceptable, and more than 83 percent indicated they would buy the product.

Economic impact

The outlook for more innovative and effective crawfish processing technology and byproduct recovery is promising. With enforcement of pollution laws to protect the environment, crawfish processors have shown an increasing interest in using undersized crawfish. This would minimize pollution problems and offset costs involved in disposal of processing byproducts and wastes, and, at the same time, maximize the processors' profits. In the long run, maximizing the use of underused, undersized crawfish will not only enhance the competitiveness of the Louisiana crawfish industry, but also enhance the state's economic development. ■

Witoon Prinyawiwatkul, Assistant Professor, and Samuel P. Meyers, Professor Emeritus, Department of Food Science, LSU Agricultural Center, Baton Rouge, La.; R. Wes Harrison, Associate Professor, Department of Agricultural Economics and Rural Sociology, University of Tennessee, Knoxville, Tenn.

Turning minced meat from catfish frames into surimi

Most catfish is processed into fresh or frozen fillets and whole-dressed fish. Other products include steaks, nuggets and value-added products, which accounted for 21 percent of the total products sold in 1998. The dress-out yield of catfish, when processed as fillets, is about 45 percent, generating about 55 percent byproducts. Up to 75 percent of usable minced meat can be recovered from catfish frames.

Catfish processors have increasingly shown interest in converting byproducts or processing waste into edible value-added food products. Several researchers have investigated the recovery of minced meat from filleted catfish frames using a mechanical meat-bone separator. This minced meat can be further processed into surimi. Surimi has little or no flavor of the original fish and therefore can be used as an intermediate raw material for various seafood-based products.

Modified surimi processing

The basic surimi manufacturing process involves washing the mince, draining to remove excessive water, straining or refining to remove remaining skin or bones, screw-pressing to dewater, mixing the mince with cryoprotectants, packing and freezing. The estimated production cost for producing surimi from mince recovered from catfish frames using the standard commercial techniques is probably higher than that of commercial surimi made from Alaska pollock. The process to produce catfish surimi requires large volumes of water to remove blood, pigments, lipids and water-soluble proteins. Reducing the water required would lower the production cost and reduce the space required for wastewater treatment. Lower production costs would encourage catfish processors to further invest to extend the production line to include surimi.

Process modification to reduce production cost of catfish surimi is being investigated by researchers in the Department of Food Science. Two different processing techniques were applied for the washing step: (1) a traditional process—twice washing using 1 part mince to 3 parts ice-water and (2) a modified process—one washing using 1 part mince to 5 parts ice-water with 0.5 percent sodium bicarbonate. The yield (about 23 percent) of surimi products prepared from both processes was not significantly different. The modified process yielded surimi with significantly lower fat (0.02 percent) and higher protein (15 percent), however, than the standard process. The modified process also resulted in a lower whiteness index and liquid expressible drip.

Our study suggested that water volume required for catfish surimi processing can be reduced. Furthermore, all significant potential human pathogens were reduced to a non-detectable level as a result of our controlled processes used during production of minced meat and surimi products.

Improving whiteness of catfish surimi

Another major concern of surimi quality is color. Surimi processed from catfish filleted frames has inferior whiteness (55) compared to commercial surimi, which normally has a whiteness

value around 67. Appearance plays a critical role in food acceptance. Our interest was therefore to determine the feasibility of improving color whiteness of catfish surimi using titanium dioxide, a simple and economical approach. The surimi samples were thoroughly mixed with titanium dioxide and subsequently analyzed instrumentally and by consumers. Significant improvement of whiteness of surimi products over the control (no added titanium dioxide) was observed at 0.1 percent titanium dioxide. Whiteness of surimi increased with increased percentage of titanium dioxide. Adding more than 0.7 percent titanium dioxide did not significantly increase whiteness. Consumers perceived differences in whiteness when titanium dioxide was added at 0.1 percent. Acceptability of surimi was increased over the control sample when at least 0.5 percent titanium dioxide was added.

Value-added formed catfish products

Interest in developing formed catfish products from unwashed mince recovered from filleted frames has increased. Because of its poor functionality, unwashed mince may not yield formed products with desirable texture. Food-grade binders have been used to enhance the textural quality of several restructured muscle food formulations. Our work indicated that whey protein isolate, sodium alginate and waxy rice starch can serve as good binders. Restructured fish products are normally prepared by chopping surimi with whole fish muscle, salt and binders. At the Department of Food Science, we have investigated the feasibility of using either unwashed frame mince or a mixture of unwashed mince and surimi as a major ingredient in restructured catfish products. Several new formed catfish products, such as nibbles, fingers, cracker and chips, have been developed and are being tested.

In the summer of 1999, we conducted a consumer acceptance test for our novel nibble product. This product was made of minced meat and surimi, rice starch, salt, white pepper, garlic powder, parsley and sweet corn. A total of 123 consumers participated in this study. More than 60 percent of the responses indicated that consumers liked the product. About 83 percent indicated that the product was acceptable, and 70 percent would buy the product. Of those who would buy, 76 percent would be willing to pay the same price as a similar product, and 7 percent would be willing to pay more.

Impact on the catfish industry

As demand for surimi continues to grow and a global natural fishery catch declines, byproducts from catfish filleting may serve as an alternative for the surimi production. Potential exists for the development of both catfish mince-based and surimi-based products, which may, in turn, form potential new market niches that will be beneficial to the catfish industry. ■

Witoon Prinyawiwatkul, Assistant Professor; Voranuch Suvanich, Postdoctoral Research Associate; and Wanda J. Lyon, Assistant Professor, all with the Department of Food Science, LSU Agricultural Center, Baton Rouge, La.

Pond Production of Catfish: Research Focuses on Improving Yields

C.R. "Chuck" Weirich

Although many advances have been made during the rapid growth of the catfish industry, today's culture practices are about the same as those developed over 25 years ago. Also, most ponds used to grow catfish are larger than 10 surface acres. The use of outdated or unrefined culture practices coupled with large pond culture systems undoubtedly restrict potential fish yields. Commercial yields range from a modest 3,000 to 6,000 pounds per acre per year.

The goal of the catfish production research program at the Aquaculture Research Station is to enhance all phases of production by developing new or refined culture practices.

Catfish production cycle includes three phases

The catfish production cycle consists of three phases: hatchery, nursery pond and growout. During the hatchery phase, egg masses procured in the spring are transported to a hatchery where they are incubated. At hatching, fry possess a yolk for four to five days and are called "sac fry." After yolk absorption, fry are called "swim up fry" because they swim to the surface in search of food. Swim up fry are typically held in the hatchery from two to 10 days and fed a finely ground feed before they are stocked into nursery ponds at rates up to 200,000 fry per acre. Fish are fed pelleted feeds and after 120 to 180 days are harvested and stocked into food fish



Photo by Chuck Weirich

Catfish lay their eggs in containers placed in the catfish ponds. Each mass normally contains between 10,000 to 20,000 eggs.

production ponds at rates up to 10,000 fish per acre.

Food fish production ponds are managed using single-crop or multiple-crop systems. In single-crop systems, fish are reared until reaching a market size of 1 pound to 1.5 pounds, at which time ponds are harvested, drained and refilled, and restocked to begin another production cycle. In multiple-crop systems, ponds contain fish of varying size classes. Market-size fish are selectively harvested with annual

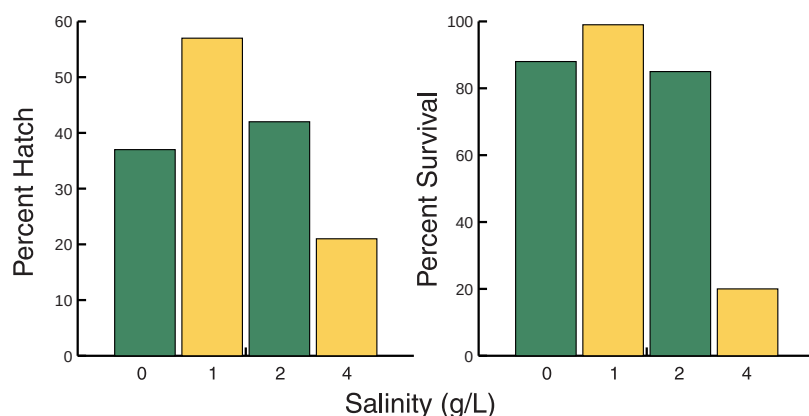
restocking of fingerlings. Thus, multiple-crop systems are constantly in production and are rarely drained. The multiple-crop system is the most common production system used in Louisiana and other catfish-producing states.

Fry and fingerling production

The following experiments have been or are being conducted to develop new and improved hatchery and nursery pond protocols to enhance production of fry and fingerlings.

Effect of salinity on production of fry and fingerlings. Although almost all catfish are produced in fresh water, several Louisiana producers have access to saline groundwater. Because little information on the effect of salinity on fry and fingerling production was available, studies were initiated in 1995. In the first study, egg masses were hatched and fry were reared for 10 days at salinities of 0, 1, 2 or 4 grams per liter. Results indicated that percent hatch of eggs and percent survival of fry were enhanced at a salinity of 1 gram per liter (Figure 1).

Figure 1. Mean percent hatch of channel catfish sac fry (left) and survival of swim up fry at different salinity levels.



C. R. "Chuck" Weirich, Assistant Professor, Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.



To harvest catfish, a seine is drawn across one end of the pond and then pulled to the other end by two tractors, one on each side. The same basic procedure is used to harvest fish commercially, only on a larger scale.

In the second study the effect of salinity on production of fingerling catfish was determined over two growing seasons (1997 and 1998). In each growth trial, fry were reared for 120 days at salinities of 0, 1, 2 or 4 grams per liter. Results revealed that while salinity had no effect on feed conversion and percent survival, weight and total yield were improved when fish were reared at a salinity of 1 gram per liter.

Effect of age of fry at stocking on fingerling production. Although most producers feed swim up fry for two to 10 days before stocking into nursery ponds,

several hatcheries in Louisiana have begun to stock sac fry to reduce feed and labor costs. To investigate this practice, a study was initiated in 1998 in which fry were stocked at two, seven or 14 days after hatching and reared for 120 days. Results indicated that there was no difference in production characteristics between age groups, suggesting that sac fry can be stocked into nursery ponds without reducing production. This study is being repeated.

Effect of hatchery diet on fry growth and fingerling production. Although a considerable amount of work has been done to develop and refine diets

and feeding practices for production of food fish, few nutritional studies have examined the hatchery and nursery pond phases of production. In 1997, two hatchery growth trials were conducted using four different hatchery diets: catfish starter alone or catfish starter supplemented with brine shrimp cysts at 25 percent, 50 percent and 75 percent of the total diet. Results from both trials indicated that weight of fry increased with increased levels of brine shrimp cysts. To investigate the effect of brine shrimp cyst supplementation of hatchery diets on fingerling production, fry from the second hatchery growth trial were reared for 120 days. Results revealed that fingerlings reared from fry fed hatchery diets supplemented with brine shrimp cysts at 50 percent and 75 percent of the total diet were larger and had a higher total yield than fish fed other hatchery diets (Figure 2).

Enhancing production

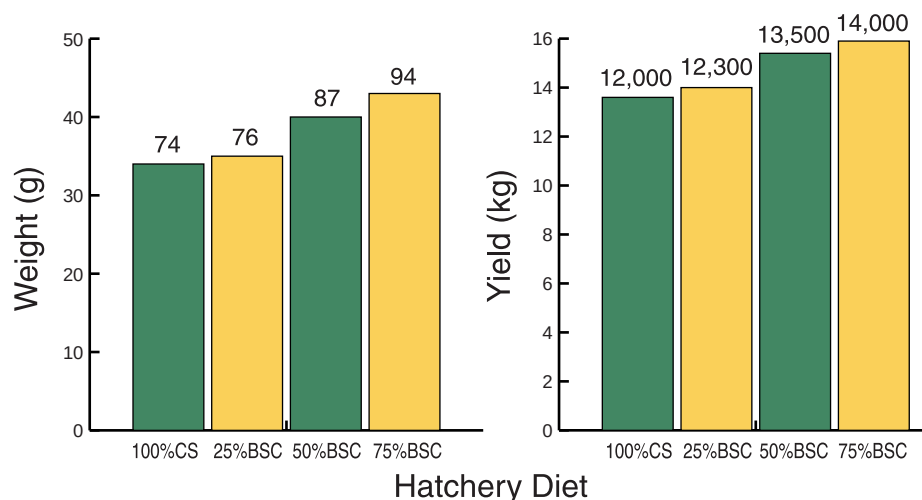
The following experiments have been or are being conducted to develop new or improved culture practices to enhance production of food fish.

Effect of salinity on production of food fish in multiple-crop ponds. A three-year study was begun in 1998 to evaluate the effect of salinity on production of food fish. Three salinity levels are being evaluated: 0, 1.5 and 3 grams per liter. Fingerlings are stocked each winter and food fish are harvested each spring and fall. Data are maintained on production characteristics and will be analyzed at the study's conclusion. Results from the two harvests conducted thus far show that total yield of fish has been higher in ponds containing low levels of salinity, but differences at this time are not statistically significant.

In addition, a study is being conducted to determine the effect of salinity on selected blood parameters and health status of fish. Another study will evaluate the effect of salinity on phytoplankton, off-flavor compounds and off-flavor status of fish.

Effect of temporarily sequestering fingerlings on production of multiple crop pond. Although most production manuals recommend that fingerlings 6 inches or longer should be stocked for growout, most producers have access to fingerlings only 2 inches to 4 inches long. It seems reasonable to assume that smaller fingerlings would not only take longer to reach harvestable size, but also may be more prone to competition and

Figure 2. Weight (left) and yield (right) of catfish fingerlings originally fed different hatchery diets (CS=catfish starter; BSC=brine shrimp cysts) then reared for 120 days. Values above bars are projected pounds/1,000 (left) or pounds/acre (right).



cannibalism from larger fish in multiple crop ponds.

One way to increase production in multiple ponds stocked with small fingerlings may be to sequester fingerlings temporarily to allow additional growth before release. In 1997, a study was completed in which fingerlings were stocked into ponds containing equal numbers of larger fish. Ponds were open or contained cages for sequestration. Fingerlings were held for 120 days before being released. At the end of the growing season, ponds were harvested. Results indicated that although survival of fingerlings was not improved by sequestration, weight of both sizes of fish and total yield were higher for ponds which had contained cages. To investigate this topic further, a three-year study was initiated in 1998. ■



Smaller catfish escape through the mesh of the seine until they are large enough to be harvested.



Catfish are removed from the seine and placed in baskets for weighing and counting. From left are Dan Ashe, research associate, Cliff O'Neal, graduate student, and Wei-Bing Yan, research associate. In front is Charles "Chuck" Weirich, assistant professor at the Aquaculture Research Station.

Potential for the Partitioned Aquaculture System in Louisiana

Robert P. Romaine and Christian Balnath

The most serious problems that face catfish farmers include losses from birds and diseases, off-flavor and the shortage of labor to harvest fish. These problems have been magnified in recent years as fish farmers have increased stocking and feeding rates to keep ahead of increasing expenses and competition.

Control options for bird predators are limited, especially for federally protected migratory waterfowl, such as the double-crested cormorant, herons and egrets. Diseases such as enteric septicemia catfish (ESC) and columnaris not only can devastate profits but create the need for additional costs in control and prevention. Blue-green algae that temporarily impart a musty, unacceptable flavor in fish flesh limit sales and hinder cash flow. They cause the additional cost of having to feed the fish while holding them until off-flavor problems subside.

A decade ago, aquacultural researchers David Brune, John Collier and Tom Schwedler at Clemson University in South Carolina developed a new concept to minimize problems with birds, water quality and off-flavor, disease and harvest. The unit was called a Partitioned Aquaculture System (PAS). The PAS integrated intensive culture of fish in rectangular units, or raceways, commonly used in mountain states to grow cold-water fishes such as rainbow trout. The system incorporated sanitary engineering principles using a pond or lagoon to reduce solids and soluble wastes.

With financial support from the Louisiana Catfish Promotion and Research Board, Louisiana Agricultural Experiment Station (LAES) scientists constructed a 0.75-acre experimental demonstration PAS unit at the Aquacul-

PAS Potential:

- Saves labor costs because a computer monitors the system. Labor shortage is one of the key constraints in aquaculture production.
- Helps prevent bird predation. Birds can significantly cut into a producer's profits. The netting on top of the raceways prevents this. It would be too costly to cover a regular pond.
- Allows for an efficient way to feed the fish and to medicate the fish in case of disease, since they are in a confined area.
- Allows for efficient harvesting because of confinement to a smaller area.
- Allows for the growing of different sizes and ages of fish in the raceways.
- Helps prevent off-flavor in fish because it is easier to control algae growth. The shallow water-flow areas, only 2 feet deep, allow the good algae to grow unhindered by the "bad" algae that thrive better in deeper water uninhibited by sunlight. Also, one of the confinement areas can be used for algae-eating fish.

ture Research Station to investigate the economic feasibility of using PAS technology in Louisiana.

PAS concept and design

The system capacity of conventional open catfish ponds is about 5,000 pounds per acre, requiring about 2 horsepower per acre of mechanical aeration to support the 100 pounds or more per acre of feed supplied to the pond daily. Yields of catfish from open ponds that exceed 5,000 pounds result from multiple cropping and re-stocking programs during the production season. In contrast, PAS technology allows

production capacity to approach 20,000 pounds of per acre per year with feed conversion ratios (weight of feed:weight of fish produced) near 1.5:1 through algal management and waste removal.

The PAS has four basic components: the fish culture unit (raceway), the settling sump, the paddlewheel mixer and the open pond (Figure 1). The raceway is separated from the open pond to facilitate better management of fish production and removal of solid and soluble waste produced by the fish. Water flow through the raceway and open pond is maintained with a low head paddlewheel mixer. A settling basin (sump), located at the outlet of the raceway(s), captures and concentrates fish waste for removal from the system. The water leaving the raceway and sump flows through the open pond, which serves as a waste treatment unit to remove soluble metabolic waste. The recirculating PAS does not require amounts of makeup water beyond the normal volumes needed to meet evaporation and seepage losses in conventional ponds.

Fish Confinement System (Raceway). The raceways are gated, rectangular troughs, 4 feet deep, that can be constructed from concrete, lumber and fabric, or other materials, and the square footage generally ranges from 2.5 percent to 4 percent of the open pond area. Two parallel raceways are used, a larger unit for the principal target species (channel catfish) and a smaller raceway reserved for aquatic organisms that consume algae. Water quality entering and leaving the raceway is monitored continuously by oxygen, temperature and pH sensors connected to a computer that can control the rotational speed of the paddlewheel mixer and operate supplemental aerators.

Settling Sump. The sump, which is 6 feet deep, accumulates solid waste from the fish and other sources, such as "dead" algae. The solids are removed periodically by automatic or manual vacuum to reduce oxygen demand, thereby increasing fish carrying capacity.

Robert P. Romaine, Resident Director and Professor, and Christian Balnath, Research Associate, Aquaculture Research Station, LSU Agricultural Center, Baton Rouge, La.

Partitioned Aquaculture System (PAS)

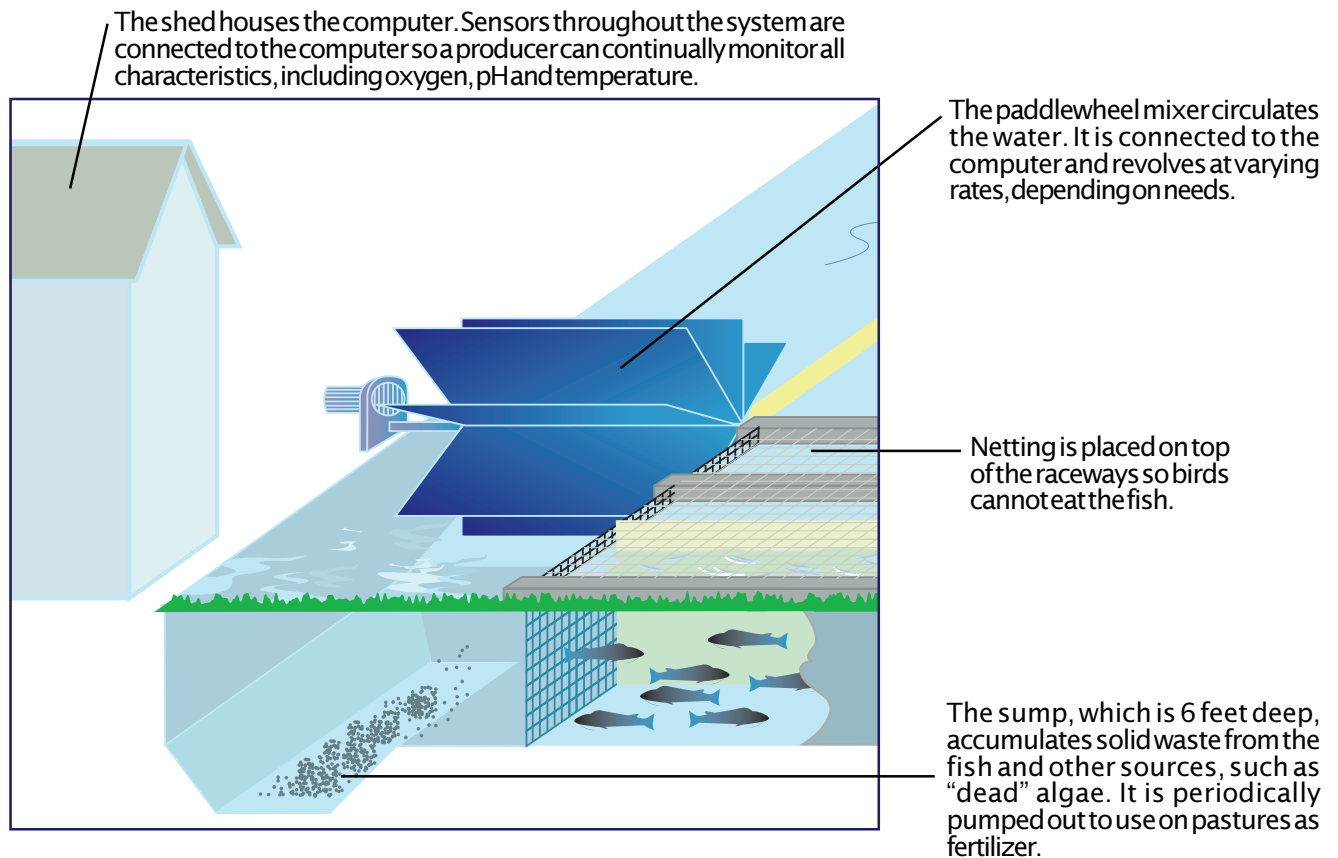
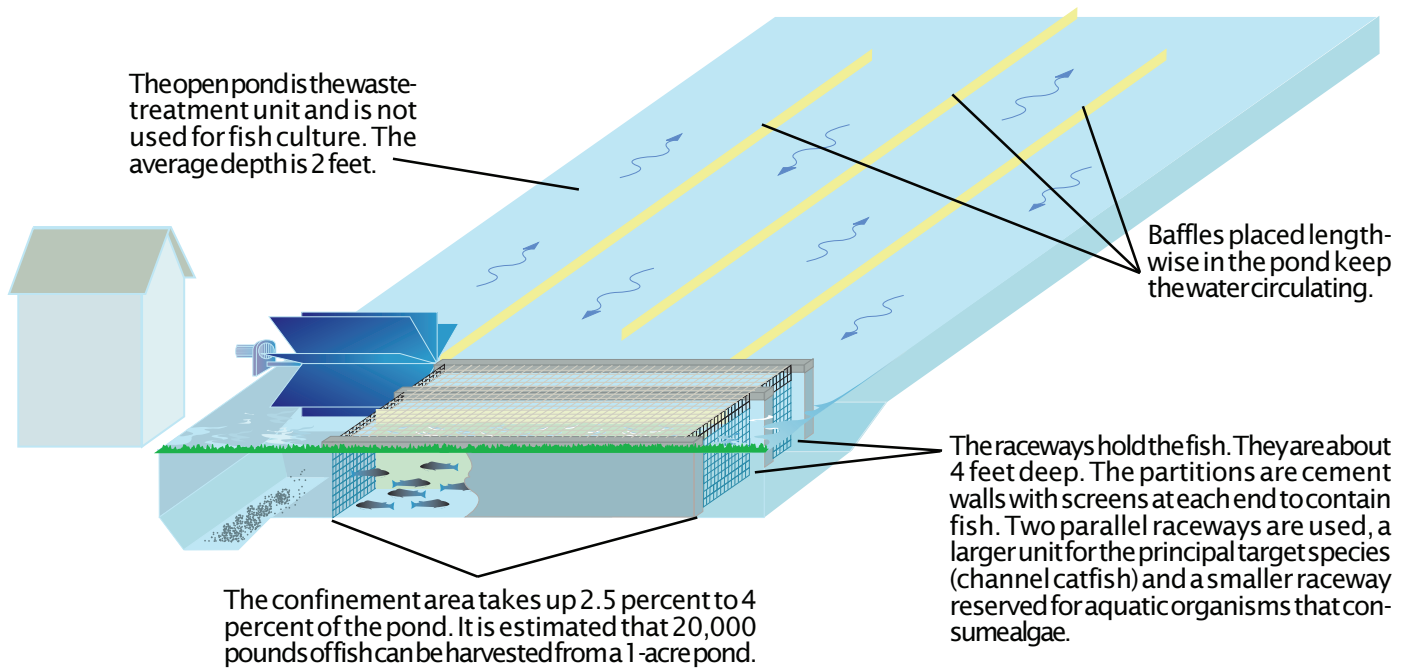


Illustration by Elma Sue McCallum



A 3/4-acre Partitioned Aquaculture System was built at the Aquaculture Research Station in 1999. Robert Romaine is in charge of research on the use of this technology.

Nitrogen and phosphorus recovered from the waste are applied to pastures.

Paddlewheel Mixer. The paddlewheel mixer is the primary control device. Designed to rotate at 1.5 revolutions per minute (rpm) to 3 rpm, it produces a water flow of 0.15 feet to 0.3 feet per second (fps) in the open pond. A computer control system regulates water velocity and reduces oxygen loss from super-saturated levels by varying the rotational speed of the mixer from information provided by the oxygen sensors in the raceways.

Open Pond. The open pond is the waste-treatment unit and is not used for fish culture. The shallow (1.5 feet to 2.5 feet) pond is subdivided into channels by internal baffles. This allows for uniform mixing of the water, optimum penetration of sunlight and maximal algal growth. Algae in the open pond removes toxic ammonia from the water and optimizes dissolved oxygen production. Algal blooms followed by algal die-offs are minimized, thus stabilizing system water quality.

Filter-Feeding Fishes. At fish production levels exceeding 9,000 pounds per acre, filter-feeding fish, such as tilapia, shad or silver carp, and freshwater mussels, must be included in the PAS to consume microscopic algae. Algal consumption by a filter-feeding fish reduces algal biomass and maintains high rates of algal growth, thus improving water quality (oxygenation and

ammonia reduction), reducing potential for off-flavor and recapturing some of the nitrogen and phosphorus excreted by the catfish into the flesh of the filter-feeding species. To optimize harvest of algae, filter-feeding fish are contained in the smaller parallel raceway. Biomass of the filter-feeding fish is maintained at 20 percent to 25 percent of the target species biomass, at season's end, to achieve effective algal control. A 300 percent to 600 percent increase in water treatment capacity over conventional methods can be achieved as a result of increased algal growth combined with algal harvest by filter-feeders and water mixing, thus increasing fish production capacity over that of conventional ponds.

Advantages, disadvantages

PAS technology incorporates the best features of earthen ponds, the least expensive form of aquaculture production technology, while simultaneously reducing many of the problems of typical pond systems. Because the fish are intensively cultured in a raceway of small area (normally 4.5 percent or less of the total pond system area), the raceway can be covered cost-effectively to protect the fish from bird predation. In addition, therapeutic drugs and chemicals for disease treatment can be administered at reduced cost because of less water volume to treat. The inclusion of a filter-feeding fish or other aquatic organism reduces the potential for off-

flavor by removal of odorous species of blue-green algae. Fish size can be managed by grading, and less labor is needed for harvesting fish than is required for conventional ponds. The system also results in lower costs for utilities and chemical use. Pond water is continuously re-used and purified, thereby conserving water resources and reducing the effects of water discharge on receiving waterways. Additionally, the PAS can be used to cultivate any aquatic species amenable to high density culture in a confined raceway, such as channel catfish, blue catfish, blue catfish and channel catfish hybrids, hybrid striped bass, ornamental carp, red drum, hybrid bluegill and marine shrimp. Because highly intensive fish culture systems such as the PAS tend to push environmental production capacity, the danger of catastrophic failure exists.

Disadvantages include the potential for greater incidence for disease because of fish density. Mechanical failure of the paddlewheel mixer or failure of supplemental aerators to activate during critical periods, such as power outages, could cause massive fish loss from oxygen depletion. One of the best filter-feeding fishes now available for algal control, *Tilapia nilotica*, is not permitted for outdoor culture in Louisiana. In addition, detailed economic data on capital construction costs and cost and returns are not known.

Scientists at the Aquaculture Research Station are working cooperatively with a commercial catfish producer in north Louisiana to evaluate the biological and economic feasibility of a 2-acre PAS built for evaluation of catfish fingerling production. The computer-assisted water quality monitoring and control system was developed and installed by Aquaculture Research Station researchers in cooperation with Kiel University, Germany.

Time will tell if the PAS will revolutionize the way channel catfish or other commercially important species are cultivated. But aquaculture researchers in the LAES will work closely with Louisiana's aquaculture producers to make the PAS a commercially viable option to consider for future economic development. ■

Price and yield risk in catfish aquaculture

Many of the problems in the catfish industry are exacerbated by the uncertainty producers face when planning their operations. The sources of risk are numerous, ranging from weather fluctuations and bird predation to regulatory policy. If ignored, each source of risk can adversely affect profitability. Because producers have little opportunity to influence output and input prices, their ability to manage risk is often severely tested. To incorporate risk into their decision-making process, farmers need information. But since their industry is relatively new, there is little information about risk. This study examined the impact of price and yield variability on the profit variability of catfish production.

Two types of data were needed: yields (pounds of catfish produced per acre) and prices (for feed and marketable catfish). Catfish yields have not been systematically measured, and some of the information needed to calculate yield has only recently been collected. As an alternative, this study incorporated simulated yield data validated using expert estimates of typical farm yields. Weather was selected as the force behind simulated yield variability because channel catfish feeding varies significantly with temperature and ceases when water temperature drops too low.

Yield distributions were simulated for three different farm sizes and two different culture systems. Farms were categorized by size into small (160 total acres), medium (320 total acres) and large (640 total acres). Channel catfish are usually cultured as food fish by one of two methods: the multiple-batch system, where multiple-size fish are cultured within the same pond, and the single-batch system. Simulations generated information on total production, average annual yields and the amount of feed required for each simulated yield.

Prices paid to U.S. catfish producers for the 1970-1997 period were obtained from the National Agricultural Statistics Service. Annual catfish feed prices were obtained from secondary sources for the 1977-1998 period (Mississippi Cooperative Extension Service Fact Sheet).

Resulting net returns distributions indicated that the most risk efficient production system for catfish is either single or multiple batches on large farms. Single-batch production system on small farms, a

common operational scenario for marginally viable producers, was the most inefficient technology/size combination. These results confirm the anecdotal evidence that sophisticated farmers choose to work with the multiple-batch production systems in order to have a steady cash flow through the year and avoid losses caused by unpredictable circumstances.

The relationship between farm size and risk efficiency may become even more important in the future if the aquaculture industry encourages the private sector

development of revenue insurance contracts for producers. If accurate yield, price and net return distribution information is available, then private insurers can, in principle, create the risk-adjusted premium and deductible schedules necessary for the establishment of a private insurance market. ■

Patricia Soto, Graduate Research Assistant, and Richard F. Kazmierczak Jr., Associate Professor, Department of Agricultural Economics and Agribusiness, LSU Agricultural Center, Baton Rouge, La.

EXAMPLE BUDGET

	Farm Size (Total Acres)		
	160 acre	320 acre	640 acre
INCOME			
Catfish yield	700,000	1,420,000	2,845,000
Catfish price (cents/lb.)	77	77	77
Income	539,000	1,093,400	2,190,650
Income per water acre	3,850	3,850	3,850
OPERATING COSTS			
Fixed operating costs			
Repairs and maintenance	12,000	20,000	36,000
Pond renovation	7,200	13,000	23,000
All fuel (electricity, diesel, gas & oil)	18,698	37,995	76,000
Chemicals	485	985	2,000
Telephone	2,000	2,500	3,100
Water quality	0	450	2,000
Fingerlings	42,000	85,200	136,560
Labor	36,660	85,000	182,963
Management	21,000	35,000	60,000
Harvesting/hauling	28,000	56,800	85,350
Accounting/legal	1,800	2,400	3,500
Bird scaring ammunition	1,000	2,000	4,000
Subtotal fixed operating costs	170,843	341,330	614,473
Interest on fixed operating costs	14,095	28,160	50,694
Total fixed operating costs	184,938	369,490	665,167
Variable operating costs			
Feed (ton)	770	1,562	3,129
Price of feed/ton	280	280	280
Subtotal var. operating costs	215,600	437,360	876,120
Interest on feed	17,787	36,082	72,280
Total variable operating costs	233,387	473,442	948,400
TOTAL OPERATING COSTS	418,325	842,932	1,613,567
COSTS PER WATER ACRE	2,988	2,968	2,836
FIXED OWNERSHIP COSTS			
Depreciation			
Ponds	13,608	26,046	52,155
Water supply	5,000	10,000	20,000
Office building	900	1,450	2,250
Feed storage	520	1,040	1,300
Equipment	32,533	60,517	119,012
Interest on investment			
Land	17,930	35,530	70,730
Pond construction	7,485	14,325	28,685
Water supply	2,750	5,500	11,000
Equipment	12,575	22,743	44,084
Taxes and insurance	2,000	4,000	6,000
TOTAL OWNERSHIP COSTS	95,301	181,151	355,216
COSTS PER WATER ACRE	681	638	624
TOTAL COSTS PER WATER ACRE	3,669	3,606	3,460
NET RETURNS PER WATER ACRE	181	244	390

Sustainability in Aquaculture

Rex H. Caffey and Richard F. Kazmierczak Jr.

Sustainability, a concept much discussed in the aquaculture industry, has become associated with the idea that production systems must be designed and sited with consideration of not only their economic viability, but also their long-term environmental and sociological impact. While the concept of sustainable aquaculture has some vocal critics, the industry has been promoting sustainable production as a way of integrating the expertise of producers, researchers, regulators and other interest groups into a process that will advance aquaculture's technological and economic development. Yet, while the need for sustainability is widely acknowledged, real debate remains over the proper definition and implementation of sustainable aquaculture systems. This continuing debate is largely because sustainability is an interdisciplinary concept that draws on basic and sociological science models, some of which conflict. As a result, the development of sustainable aquaculture is not only a technical challenge, but also a political and perceptual challenge.

Survey approach

A collaborative study between the Aquaculture Research Station and the Department of Agricultural Economics and Agribusiness was initiated to determine whether diverse aquaculture interests groups could collectively develop and agree on economic, environmental and social indicators of aquaculture sustainability. Once identified and weighted, these indicators were to form the basis of a numerical index that could be used to evaluate the relative sustainability of aquaculture production systems in the southeastern United States. The ultimate validity of the index as an evaluation tool hinged

on whether a consensus could be developed among a wide variety of participants in the aquaculture industry. A type of panel procedure, known as a Delphi survey process, was used in the study.

Delphi surveys are a way to develop and measure the degree of consensus among experts. A Delphi survey is considered superior to a simple expert survey or focus group because it involves a series of questionnaires (in this case, three rounds) administered to individuals in a manner that protects the anonymity of their responses and thus their ability to freely express opinions. Feedback to the respondents between survey rounds also allows participants to reevaluate their responses based on new information provided by the expert group as a whole without knowing what specific individuals have said. Both the anonymity and feedback features of Delphi surveys have been shown to lead to the unbiased convergence of expert opinion, even among groups that initially hold widely disparate views. For this reason, Delphi surveys, which were originally used in cold-war strategic defense studies, have become widely

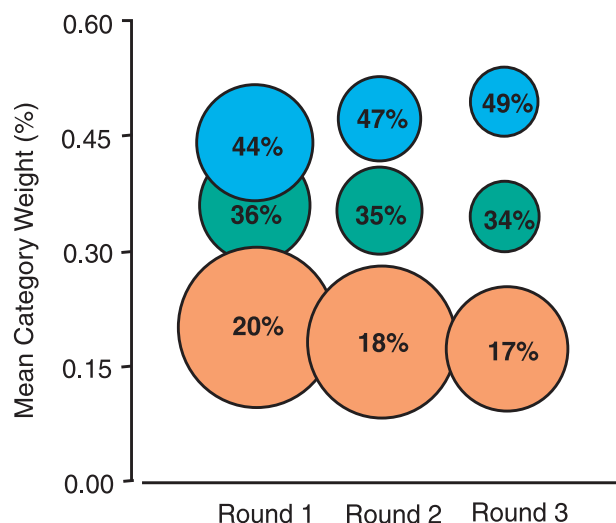
used in marketing, management and technological evaluation studies.

The experts surveyed in this study came from four different groups that have had direct involvement in the aquaculture sustainability debate: 1) aquaculture producers, 2) aquaculture researchers and extension agents, 3) aquaculture-related regulatory authorities and 4) non-governmental organizations (NGO). The states involved were Texas, Oklahoma, Arkansas, Louisiana, Mississippi, Tennessee, Kentucky, Alabama, Georgia, Florida, South Carolina, North Carolina and Virginia. Of the 163 experts who initially agreed to participate, 121 replied to the first questionnaire for a response rate of 75 percent. By the third round of the survey, the retention rate had increased to 94 percent, with 104 of the original panel participating. These response rates were higher than originally anticipated, making this Delphi survey one of the largest non-defense studies ever done.

Weighting sustainability

As part of each survey round, participants were asked to score the relative importance of the three main facets of sustainability. This information was needed not only to get participants to think about the potential tradeoffs inherent in the sustainability concept, but also for weighting the indicators that would ultimately comprise the sustainability index. Figure 1 shows the revealed weightings across the three survey rounds. In the first round of the survey, participants believed that economic considerations should make up 44 percent of the sustainability index,

Figure 1. Mean preference distribution of environmental (X), economic (Y) and sociological sustainability (Z) for rounds 1-3 of the Delphi survey. The circle size denotes the relative coefficient of variation. The smaller the circle, the greater the consensus.



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followed by 36 percent for environmental and 20 percent for social considerations. By the third survey round, participants had increased their weightings for economic considerations 5 percent while decreasing their weightings for environmental and social consideration by 2 percent and 3 percent, respectively.

More important, changing coefficients of variation (CV) across the three survey rounds suggest that the economic and environmental categories developed greater consensus on their relative importance than did the sociological category. These results were consistent with anecdotal evidence, frequently expressed by the survey participants, that aquaculture sustainability should be defined primarily by economic and environmental concerns, with social considerations given subordinate or irrelevant status. However, many NGO participants continued to hold the view that social variables should be an important part of the sustainability criteria even while recognizing the important role of economic and environmental variables.

Specific indicators

Items submitted in round 1 as potential measures of aquaculture sustainability were condensed to avoid duplication into 31 core indicators: 12 environmental, 10 economic and 9 social. The degree of agreement on individual indicators varied considerably, with the highest consensus typically accompanying those indicators with the highest revealed weighting. For example, "water usage" and "profit" had the highest mean weights and lowest CV levels, while indicators like "use of non-native species" received low mean weightings with the highest CV levels.

The average coefficient of variation fell from 63 percent to 24 percent between rounds 2 and 3, providing an indication that the Delphi survey was successful in creating a significant degree of consensus. This indication was further confirmed by nonparametric statistical analyses of the data using Friedman's Randomized

Block Design, Kendall's Coefficient of Concordance and a Distance Metric approach. Results of the nonparametric analyses indicated definite rank correlation patterns, rank convergence and rank consensus in the Delphi survey data.

MIDAS model

Having achieved a relatively strong consensus on sustainability categories and indicators, a method of integrating this information into a preliminary index of aquaculture sustainability was needed. A multi-criteria analysis framework provided the basis for this index development. The 31 indicators were combined within three linear sub-indices: environmental, economic and sociological. The contribution (weight) of each indicator was included along with a method for scoring the indicator for a given production system. The scoring system was developed in a way that allowed any indicator to add or subtract from the index the full amount of its revealed weight, depending on its score relative to statute, rule or common

practice standards. The final model form was termed Multicriteria Indicators of Delphi-Assessed Sustainability (MIDAS). The MIDAS index used 3-dimensional vector calculations to generate an index score representing the separate environmental, economic and sociological objectives of aquaculture sustainability.

MIDAS has a number of potential applications. If adequate secondary data are available, they can be used to evaluate the production-level sustainability of aquaculture under various input scenarios. In addition, they can be used to analyze the trade-offs that often occur when attempting sustainable aquaculture developments. Such evaluations have been completed and are being refined for warmwater species such as channel catfish and red swamp crawfish. Last, the MIDAS model could be used to clarify the critical economic, environmental or social issues that need further research efforts as the industry continues to move toward sustainable modes of production. ■

Ornamental ponds and water gardens



Photoby C. Greg Lutz

Ornamental ponds and water gardens are becoming increasingly popular. It is estimated that there are about 40 new ones per week in the vicinity of Baton Rouge alone. This phenomenon offers entrepreneurial opportunities for those interested in aquaculture. These ponds need to be designed and stocked. Preferable varieties of fish include goldfish and koi. These ponds also must be maintained.



From the air it is easy to spot the Aquaculture Research Station with its 146 earthen ponds. The largest in the upper right is 17 acres, and the smallest are 0.1 acre. At the bottom left are the crawfish research ponds. They are green now because rice is growing in them. Above them in the center is the Partitioned Aquaculture System (PAS) pond with its stripes created by the baffles that run through it. The office and laboratory building is on the right. Beyond the ponds and toward the horizon is the Ben Hur Research Farm, also part of the Louisiana Agricultural Experiment Station.

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