U.S. Department of Agriculture Agricultural Research Service October 2010

Agricultural Research

Aquaculture

Research Helps Fish, Producers — and You!

pages 2-20

Agricultural Research Service • *Solving Problems for the Growing World*

Supporting U.S. Aquaculture

Aquaculture provides half of the world's seafood, with about 50 million tons grown worldwide in 2006. Further, half the seafood consumed in the United States comes from aquaculture, and yet about 85 percent of that amount is imported.

Broadly defined, aquaculture is the captive rearing of any life stage of an aquatic organism and includes fish farming as well as activities like the hatching and releasing of sport fish by state agencies. The U.S. Department of Agriculture (USDA) defines aquaculture as a private-sector enterprise. The Agricultural Research Service (ARS) conducts research primarily to support production of aquatic animals that are privately owned. In 2009, Americans consumed an average of about 16 pounds of seafood per person, but less than 1.5 pounds of that was from domestic aquaculture.

Research has identified numerous beneficial effects of consuming seafood, including improved cardiovascular health in adults and neurological development in children. This research has suggested that eating the recommended two servings of fish or shellfish each week could dramatically reduce the risk of death from heart disease.

Seafood demand in the United States and worldwide is steadily increasing due to both population growth and increased demand for seafood per person. But fish and shellfish taken from the ocean are already at or above sustainable levels, so any increase in seafood supply will have to come from aquaculture.

The goal of ARS's Aquaculture national research program (#106) is to conduct research to support existing industries and provide impetus for continued development of a thriving domestic aquaculture industry that can provide U.S. consumers with delicious, nutritious, affordable, and safe seafood. Our research considers the basic principles of sustainability and quality. These include raising fish and shellfish in a manner that protects and enhances the environment and enables those along the whole value chain—from hatchery operators, farmers, feed producers, processors, and distributors—to profit and compete while producing seafood that has all the value and quality expected from U.S.-produced food.

With thousands of miles of coastline and abundant freshwater resources, the United States has the potential to be a major producer of seafood. ARS aquaculture scientists are focusing on genetic improvement, feeds and nutrition, animal health, production systems, and environmental impacts. These research initiatives and results are explored in this issue.

Major research initiatives include finding alternatives to fishmeal in aquaculture diets; improving fish and shellfish survival through vaccines, therapeutants, and breeding programs for better disease resistance; and developing production systems to reduce costs and improve profitability.

Currently, most fish feeds include ingredients like fishmeal and fish oil that come from small ocean fish, like menhaden, which are not usually consumed by humans. But demand for fishmeal and oil is high, and the supply is limited. Consequently, ARS scientists in Idaho, Alabama, Arkansas, and Florida, working with collaborators at universities and other research institutions in the United States and around the world, are developing alternative feeds that include plant- and algal-based meals and other products that will reduce the need for fishmeal and fish oil in aquaculture feeds. The ongoing Alternative Feeds Initiative—involving USDA's ARS and National Institute of Food and Agriculture and the National Oceanic and Atmospheric Administration—has focused attention on this area.

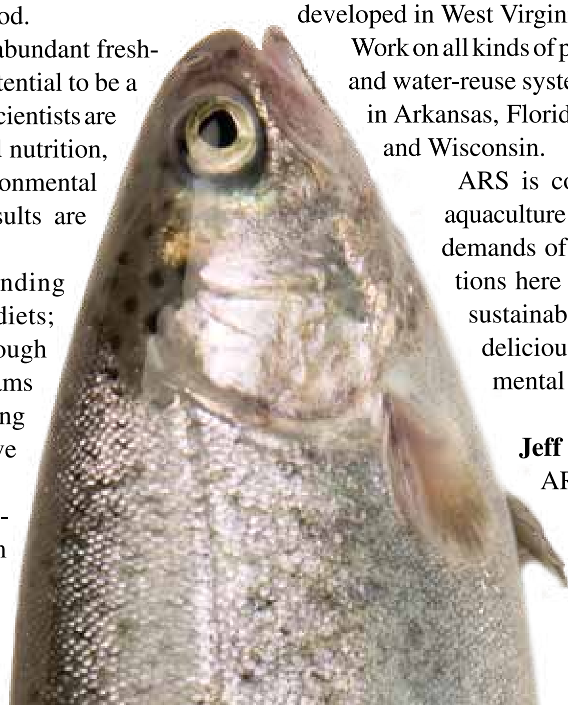
ARS scientists at labs in Alabama, Arkansas, Florida, Maine, Mississippi, Oregon, West Virginia, and Wisconsin are examining fish and shellfish health. The genetics of disease resistance, vaccine development, the host immune system, and pathogen genomes are all being investigated as ways to improve the performance and yield of aquatic systems.

Improving consistency and product quality is another important concern. In particular, treatments to avoid off-flavors in fish are being developed in Louisiana and Mississippi ponds as well as in recirculating aquaculture systems.

Sustaining production without harming the environment is critically important and a priority for the industry. Pond systems with catfish, raceway systems with trout, and net pens with salmon are several traditional production systems. Newer recirculating aquaculture technologies require less water, collect fish wastes, and offer stricter control of water quality—all benefits to the environment and the producer. Recirculating systems generally require higher energy inputs than typical systems, but they have the potential to be located in areas with less abundant water supplies and nearer to markets. ARS scientists have conducted long-term studies on recirculating systems. Tank systems using recirculating aquaculture strategies for freshwater and saltwater fish have been successfully developed in West Virginia and Florida.

Work on all kinds of production systems—ponds, raceways, and water-reuse systems—is being conducted by scientists in Arkansas, Florida, Maine, Mississippi, West Virginia, and Wisconsin.

ARS is committed to providing the tools the aquaculture industry needs to meet the consumption demands of U.S. consumers. Through collaborations here and abroad, these tools will result in sustainably raised seafood that is nutritious and delicious and supports human and environmental health.



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October 2010
Vol. 58, No. 9
ISSN 0002-161X

Agricultural Research

Solving Problems for the Growing World

STEPHEN AUSMUS (D1931-10)

Catfish several days after hatching. ARS has a better way to hatch catfish eggs (story begins on page 7).

Cover: Juvenile rainbow trout raised in a tank at the ARS National Center for Cool and Cold Water Aquaculture in Leetown, West Virginia. Scientists there have been working to breed resistance to bacterial cold-water disease into rainbow trout. Story begins on page 11. Photo by Stephen Ausmus. (D1946-4)

Agricultural Research is published 10 times a year by the Agricultural Research Service, U.S. Department of Agriculture (USDA). The Secretary of Agriculture has determined that this periodical is necessary in the transaction of public business required by law.

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RECIRCULATING WATER Helps

Laboratories in the Agricultural Research Service's aquaculture program are working well together. In developing recirculating systems for fish production, ARS labs in West Virginia and Maine are working with the Conservation Fund's Freshwater Institute (FI) in Shepherdstown, West Virginia, to find out how best to produce fish that live in cool and cold water.

Also, an ARS researcher in Stuttgart, Arkansas, has developed recirculating systems that address the needs of warm-water marine species.


PEGGY GREB (D1955-2)

At the Conservation Fund's Freshwater Institute in Shepherdstown, West Virginia, ARS engineering technician Clayton Birkett (left) and Steven Summerfelt of the Freshwater Institute collect water from a tank used to study Atlantic salmon growth and test the sample for dissolved oxygen concentration.

A (Recycling) Splash of Cold Water

A research partnership between ARS and FI has refined a new model for fish farming, one that can produce healthy fish, leave a healthy environment, and be done almost anyplace, even far from large water resources.

The research, carried out through a cooperative agreement between the U.S. Department of Agriculture and FI, has pioneered ways to best produce cool- and cold-water fish in the tanks of a water-recirculating fish farm. Says Steven Summerfelt, director of aquaculture systems research at FI, "The recirculating systems developed here have several advantages over net pens and flow-through culture systems. Because they require far less water and capture the wastes, a fish farm that recirculates its water can be located far from large water bodies and near consumers. That gets fresh fish to market faster and with less transportation cost."

Fish that require pristine water conditions were once thought to be too difficult to grow in tanks supplied with recirculating water. Trout are a prime example. Particularly sensitive to water quality, they will not eat enough feed to grow if conditions aren't right. But they are in high demand as food. So ARS and the team of problem-solvers at FI have taken a systems approach and designed fish-farming systems that address stocking of fish, waste removal, biosecurity, water-quality monitoring, and fish harvesting step-by-step, so that trout and many other high-quality market species will thrive in water-recirculating systems.

"Twenty years ago, it was believed that trout couldn't thrive in a water-recirculating system," says Summerfelt. "But we have now developed systems that maintain water clean and fresh enough for trout, salmon, and Arctic char to grow and thrive."

Summerfelt says the systems have additional advantages over traditional fish-culture technology. "They prevent fish from escaping the farm, treat waste to avoid polluting the watershed, and recapture nutrients for other agricultural uses," he says. "We have also nearly eliminated chemical and antibiotic treatments, because these nearly closed systems effectively exclude fish pathogens. Our biggest challenge has been to make these new technologies more cost-competitive against traditional net-pen, pond, and flow-through fish-culture technologies."

At this point, Summerfelt says, "specific high-value niche species are being farmed in water-recirculating systems. But we expect that domestic production of many fish species, including salmon, will eventually become competitive in domestic, large-scale, land-based, closed-containment aquaculture systems."

William Wolters, director of the National Cold Water Marine Aquaculture Center in Franklin, Maine, is impressed. They use a water-recirculating system developed at FI for Atlantic salmon.

"The recirculating systems are working very well," says Wolters. "In our area, there are limited ground-water resources, so we need this system to reuse water. We have been able to raise our fish efficiently with that limited amount of water."

In addition, research leader Caird Rexroad III, geneticist Timothy Leeds, and physiologist Gregory Weber, at the ARS National Center for Cool and Cold Water Aquaculture (NCCCWA) in Leetown, West Virginia, maintain a broodstock-development program in which fish are produced in recirculating systems developed by Summerfelt. In 2008, a new facility based on this technology was completed, and it now houses the entire life cycle of the NCCCWA broodstock, which are evaluated for production efficiency.

Recirculating systems from FI use as little as 4 percent new water each day, which means that a complete water exchange takes place once every 25 days. This miserly use of water, an environmental plus, also allows recirculating fish-farm systems to be located in many places where traditional aquaculture wouldn't work.

NCCCWA and FI scientists are also determining how different fish feeds affect fish performance and health and water quality. Open-formula, fishmeal-free trout feeds, developed by ARS fish physiologist Rick Barrows at the Hagerman Fish Culture Experiment Station in Hagerman, Idaho, and by his colleagues, result in fish performance equal to that obtained with current industry-standard diets containing much higher levels of fishmeal. Furthermore, the fish waste—a major source of phosphorus and nitrogen pollutants—is firmer, allowing it to settle quickly out of the system. Waste removal is important for maintaining water quality.

"Dr. Barrows's alternative diets work well in recirculating systems. The fish grow just as fast, but they have better fin condition, and we can maintain excellent water quality," says Summerfelt.

PEGGY GREB (D1956-1)

Thomas Waldrop (left) and Steven Summerfelt, at the Conservation Fund's Freshwater Institute, sample Atlantic salmon in a 40,000-gallon closed-containment system.

PEGGY GREB (D1953-1)

ARS agricultural engineer Timothy Pfeiffer (left) and ARS technician Todd Lenger observe growth of Florida pompano and adjust dissolved oxygen inflow in a multiple-tank hatchery system.

Frisky Warm-Water Marine Fish

Further south, other ARS scientists have also successfully developed recirculating systems. Agricultural engineer Timothy Pfeiffer, based at the Harry K. Dupree Stuttgart National Aquaculture Research Center, in Stuttgart, Arkansas, conducts his research at the Aquaculture Park of the Florida Atlantic University/Harbor Branch Oceanographic Institute in Fort Pierce, Florida. He has evaluated recirculating systems for various marine fish, including cobia, red drum, sea bass, and pompano. Pfeiffer used Florida pompano in his most recent studies to determine the fish's response to low-salinity water at high density in recirculating systems. Pompano is a high-value, fast-growing fish found in waters of the U.S. eastern seaboard from Virginia to Florida. They can attain a length of up to 12 inches in the first year of life.

Pfeiffer concentrates on evaluating various components of the recirculating system and the efficiency of the system's unit operations—solids removal, aeration, carbon dioxide removal, biofiltration, and disinfection. Different system components and operational strategies affect the system's water quality and, ultimately, the mass of fish that can be cultured in the tanks.

In recirculation systems, energy is a major cost factor, so research to reduce the energy demands is critical. One energy-intensive operation being examined for modification is water pumping. Recirculating aquaculture systems mainly use centrifugal pumps to move water throughout the system treatment components and tanks, which requires high energy input.

One of Pfeiffer's studies of recirculating systems for pompano production evaluated high-head versus low-head pumping systems to determine energy requirements and how many fish could be maintained within each system. High-head systems refer to high-pressure centrifugal pumps, and low-head systems refer to low-pressure axial flow propeller pumps.

"We found that use of low-head pumps with proper system plumbing, minimal bends in the pipe, and larger diameter pipes requires less energy, which can make the enterprise more sustainable," says Pfeiffer. "A properly designed low-head system with an axial flow pump can use up to 30 percent less energy than a high-head centrifugal pump system."

Stocking density of fish was also investigated. Traditional pond culture without water recirculation generally operates at densities of 0.005 to 0.007 pounds of fish per gallon of water. "We are able to maintain as high as 0.5 pounds of fish per gallon of water," says Pfeiffer. "We want to push our capacity to 0.75 pounds per gallon, on par with the most efficient systems known, and we expect to achieve that capacity with some design modifications."

The tanks were 10 feet in diameter and 4 feet deep, holding about 2,000 gallons of water. "All the systems are automated to monitor and control oxygen concentration in the tanks and feed fish," says Pfeiffer. "These systems can be applied to either freshwater or saltwater fish culture."

In addition to investigating recirculating systems for the grow-out phase, Pfeiffer also investigated recirculating-system designs for hatchery production. "We constructed two hatchery multi-tank systems that use air to lift and circulate water in the tank," says Pfeiffer. "The air-lift pumps produce bubbles that lift the water as they travel upward through the water column, achieving circulation, aeration, and degassing all in one action."

Recirculating aquaculture systems have a bright future. Their advantages—a controlled environment that optimizes growth and protects against pathogens and disease, a reduced water requirement, and the ability to remove and reuse animal wastes—make these systems able to provide a continuous supply of fresh, healthy fish to those in and near land-locked areas as well as those near the coast. As energy efficiency increases and the control offered by recirculating systems becomes more valuable, these technologies will grow in use.—By **Sharon Durham,** ARS.

This research supports the USDA priority of ensuring food safety and is part of Aquaculture, an ARS national program (#106) described at www.nps.ars.usda.gov.

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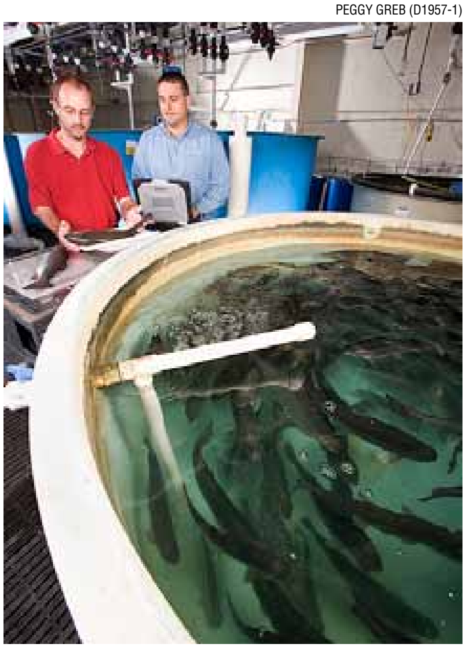
PEGGY GREB (D1954-1)

At the Florida Atlantic University/Harbor Branch Oceanographic Institute, Todd Lenger (right) and professor Paul Wills maintain and adjust movement of the system's biofilter media (the small, round objects in the water). In the background, facility systems manager Richard Baptiste monitors dissolved oxygen concentration.

PEGGY GREB (D1957-1)



Geneticist Tim Leeds (left) and research leader Caird Rexroad III use a transponder tagging system to record length and weight of trout.

A Better Way To Hatch CATFISH EGGS

Agricultural Research Service scientist Les Torrans has spent the bulk of his career in the Mississippi Delta looking for ways to help commercial farmers grow a better catfish or develop more efficient ways of feeding and harvesting them.

Now, with the U.S. farm-raised-catfish industry struggling in the face of cheaper foreign imports and rising production and feed costs, the scientist—based at the Catfish Genetics Research Unit of the Thad Cochran National Warmwater Aquaculture Center at Mississippi State University (MSU), in Stoneville—is focusing on new field research he thinks could provide some help.

In just the last year or two, Torrans says, the number of catfish hatcheries in his portion of the Delta has declined from 30 to just a dozen or so.

While growers have known for as long as there have been commercial catfish farms that aeration of ponds was critical for fish survival, Torrans provided empirical evidence on exactly how oxygen levels affect feeding and production in ponds. His research has produced new oxygen-management recommendations for ponds that can save energy and improve production.

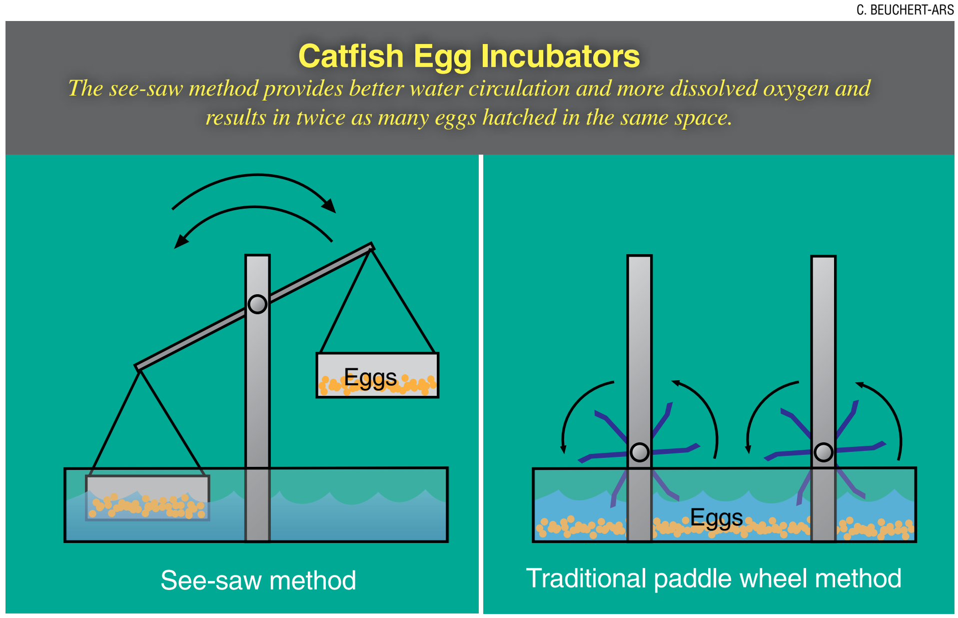
More recently, Torrans and MSU's James Steeby developed specific dissolved oxygen recommendations for catfish hatcheries. Data they collected on catfish egg and fry metabolism showed that most hatch variations are caused by insufficient dissolved oxygen in the water. Poor water circulation around and through egg masses, especially at high egg-loading rates, compounds the problem, according to Steeby and Torrans.

Now Torrans is applying his mechanical skills to building a high-intensity catfish egg incubator he calls a "see-saw." Torrans says the device will provide a better mix of dissolved oxygen for vulnerable catfish eggs by dipping the egg masses, much like a tea bag being dipped in water, then lifting them completely out of water before dipping them again. As long as the egg masses stay wet, they are able to exchange gas across the chorion (egg shell). Oxygen in air is much more abundant than oxygen in the water, so this innovative see-saw device takes advantage of the water dipping to maintain the developing eggs' moisture while using the air for oxygen delivery.

STEPHEN AUSMUS (D1929-5)

Catfish farmer Bobby Jones (left) and his father Robert A. "Shorty" Jones, of Needmore Fisheries LLC, observe the "see-saw" egg incubator with co-developer fish biologist Les Torrans (center). The two farmers plan to replace their traditional paddle-type incubators (seen on the right, behind Jones) with the new see-saw device before next spawning season.

C. BEUCHERT-ARS



"It looks like we can hatch more than twice as many eggs with the see-saw in the same space and use half as much water as with the traditional paddle-wheel method," says Torrans.

"What we're seeing is an industry in trouble in Mississippi, Alabama, Arkansas, and Texas, where more than 90 percent of the nation's catfish are grown," he says. "If we can get more dissolved oxygen to the eggs, more will hatch. That would obviously be a boost to farmers. We have to make our domestic production as efficient as possible."—By **Chris Guy,** ARS.

This research is part of Aquaculture, an ARS national program (#106) described at www.nps.ars.usda.gov.

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Finding Alternative FISH FEEDS for

Imagine you are invited to a buffet lunch and the only thing on the menu is a food you don't enjoy. How much do you think you will eat? While it may qualify as a wonderful, nutritious food, you won't necessarily partake.

Now, imagine you are a fish in a tank full of feed you won't touch.

Reducing reliance on captured marine fish proteins and oils for fish feeds is an important goal in aquaculture. But fish are picky about their feed, and fish nutritionists need to find nutritious feeds, low in fishmeals and oils, that fish will eat.

A Growing Demand

Feed provides the nutrients required for building and sustaining life. If fish don't eat well, they won't grow and thrive. Commercial fish farms rely on feeds that now include fishmeal as a major source of protein and oil. The protein- and lipid-rich feed pellets used for farmed fish have traditionally been made in part from small, bony fish species, like menhaden, herring, and capelin.

Pet food and diets for swine and poultry have also traditionally used fishmeal and oil. And as more people around the globe turn to fish as a source of lean protein, they drive the growth of aquaculture worldwide; aquaculture now supplies half of the seafood produced for human consumption.

Thus, the demand for proteins and lipids for fish diets has increased while the supply of marine fishmeal has not increased, causing more pressure and price increases on the limited supply of fishmeal. Replacements for fishmeal and fish oil are needed to support sustainable aquaculture.

Agricultural Research Service (ARS) aquaculture scientists are working to develop fish feeds that don't include fishmeal. Since November 2007, the U.S. Department of Agriculture (USDA) and the National Oceanic and Atmospheric Administration have been engaged in the Alternative Feeds Initiative to accelerate development of other feeds for aquaculture. The initiative's purpose is "to identify alternative dietary ingredients that will reduce the amount of fishmeal and fish oil contained in aquaculture feeds while maintaining the important human health benefits of farmed seafood."

STEPHEN AUSMUS (D1228-11)

Fish nutritionist Marty Riche feeds juvenile Florida pompano during studies to determine appropriate feeds and feeding-management practices for profitable inland production of saltwater fish.

Developing alternative ingredients that are produced in sufficient quantities to become standard components of diets is a key research priority, requiring understanding of an ingredient's nutritional value, its ability to blend with other ingredients, its effect on pellet stability, and, of course, its appeal to fish.

Different Fish, Different Food

Different species of fish not only have different nutritional requirements, but they also seem to have different palate requirements. In Hagerman, Idaho, ARS fish physiologist Rick Barrows is tasked with formulating and manufacturing feeds for several fish species, including trout, salmon, white seabass, and yellowtail. Barrows and his ARS collaborators have developed many different formulations for these fish depending on their stage of development, from fry (baby fish) to adults. Barrows produces the feed himself using a piece of food-manufacturing equipment called a "cooking extruder."

"My colleagues and I are open to looking at a variety of ingredients for fishmeal replacement, including plants, animal processing products, and even single-cell organisms like yeast and bacteria," says Barrows. "We conduct not only growth studies but also palatability studies to ensure fish will eat the nutritious feed."

Feeding Salmon

William Wolters, research leader and director of the ARS National Cold Water Marine Aquaculture Center in Franklin, Maine, is collaborating with Barrows to develop diets for Atlantic salmon using concentrated plant proteins. Protein levels in most grain and oilseed sources are low and need to be concentrated to reach the high protein requirements of fish. Current studies are evaluating six experimental diets, containing combinations of alternative proteins, and a fishmeal control diet being fed to fish in 15 tanks with automatic feeders.

"While the studies are still ongoing, it certainly seems that the modern alternative diets work better than previous alternative diets," says Wolters. "When top-performing low-fishmeal feeds in these studies are identified, they will be further evaluated in two separate studies."

Salmon spend 1½ years in the hatchery and then 1½ to 2½ years growing to adult size. According to the Maine Aquaculture Association (MAA), there are about 1,300 acres of marine waters leased in the state for aquaculture purposes—600 acres for finfish. Salmon is a large commodity in Maine aquaculture—annually producing between 25 and 35 million pounds.

"The issue of substitutes for fishmeal and oil as feed ingredients is a very important one that cuts across many finfish species," says MAA executive director Sebastian Belle. "The work that Dr. Wolters is doing on salmon feeds, in cooperation with Dr. Barrows, is groundbreaking and, if successful, will be very helpful to domestic growers in meeting the challenge of limited fishmeal and oil supplies. Worldwide, these supplies are limited, and as aquaculture increases we must find alternative feed ingredients that satisfy the fishes' nutritional needs while resulting in a product with the appropriate nutritional qualities for humans."

Providing Nutrients for a Hungry Species

In Florida, there is interest in rearing saltwater Florida pompano in low-salinity water in order to diversify production to inland fish farms, bringing pompano fish stocks closer to consumers. Pompano, an active, fast-growing fish, is one of Florida's highest valued fish. ARS fish biologist Marty Riche, at the Harry K. Dupree Stuttgart National Aquaculture Research Center's facility in Fort Pierce, Florida, is developing the alternative feeds for this species.

STEPHEN AUSMUS (D1429-3)

Fish nutritionist Rick Barrows examines flax oil that will be infused into pellets for rainbow trout feed.

Riche uses various ingredients, like corn gluten meal, soy proteins, and poultry-processing coproducts, to develop feeds containing less fishmeal.

"Pompano are voracious eaters—especially larvae and juveniles," says Riche, "so much so that they will eat beyond being satisfied. This eating behavior is reflected in larval and juvenile growth."

Barrows, Riche, and other ARS researchers are developing a nutrient-availability database of different ingredients that have potential to replace fishmeal. "Nutrient availability" describes how much of the ingredient is available to the animal for sustenance. "There are currently 17 ingredients in the database for pompano," says Riche.

While fishmeal can largely be replaced without harming fish health, fish oils are not so easily replaced. "Plant oils are now being identified that could possibly replace or substantially reduce the use of fish oils in feed products," says Riche.

R. BARROWS (D1942-1)

Fishmeal-free diet for California yellowtail containing 30 percent spirulina.

When we formulate a new feed, we test trout first in our palatability studies, since they are aggressive eaters. If trout eat the feed, we then test it on other species."

This work is pivotal to the feed-manufacturing business. According to Barrows, "ARS is exploring ingredients and combinations and developing gateway formulas to prove the concept of fishmeal-free feeds. This eliminates the risk commercial feed developers would usually carry. Now feed companies can use ARS fishmeal-free feed as a basis for their own fish-feed formulas."—By **Sharon Durham,** ARS.

This research is part of Aquaculture, an ARS national program (#106) described at www.nps.ars.usda.gov.

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Serving Up Algae

Algae may have the potential to replace fishmeal completely in some fish feed and perhaps replace some of the fish oils. Under a cooperative research agreement with Kent Bioenergy of San Diego, California, Barrows is investigating the use—in fish feed—of protein coproducts that result from biodiesel production from algae.

Bioenergy production from algae is a growing industry: A few years ago there were 12 companies producing ethanol this way; last year there were 100, and 350 are projected to be in existence by the end of 2010.

Barrows and corporate collaborator Carbon Capture Corporation in Imperial Valley, California, are using algae to create fishmeal-free diets for California yellowtail and white seabass. "We are finding that algal feeds could be competitively priced, contain fewer contaminants, result in fewer nutrients in fish effluent, and be sustainable," says Barrows.

Sometimes palatability is a hurdle when feeding a new type of feed to fish. "But," says Barrows, "it appears that dried algae as fish feed actually increases fish appetite.

Microalgae grown in raceways at Kent BioEnergy, in San Diego, California. The microalgae will be used in a variety of applications.

JIM DEMATTIA (D1941-1)

Containers of algae cultures at the Carbon Capture Corporation in Caliptria, California. The algae will be used to inoculate large production ponds.

Keeping Aquaculture Fish HEALTHY

JULIA PRIDGEON (D1938-1)

Immersion vaccines have been used for decades, but current work on developing alternative methods of vaccine delivery through feeding is promising. Here, molecular biologist Craig Shoemaker (left) and microbiologist Phillip Klesius demonstrate a technique where channel catfish are immersed in water containing the modified live *Streptococcus iniae* vaccine.

Keeping aquatic animals healthy in tanks, ponds, and cages is a critical aspect of fish and shellfish farming.

ARS scientists are researching various aspects of fish health, including genetic-based resistance to pathogens, treatments to cure diseases, and vaccines to prevent them.

Cold-water Disease Targeted

At the National Center for Cool and Cold Water Aquaculture (NCCCWA) in Leetown, West Virginia, one of the diseases researchers are investigating is bacterial cold-water disease, caused by the bacterium *Flavobacterium psychrophilum*. This disease is a longstanding problem in trout aquaculture, and methods for its control are limited. The disease was first described in 1948, and it occurs in salmon, trout, and a few other species. It often arises at low temperatures, and infected fish may display a range of clinical signs, including large, open lesions on the tail area as well as systemic infection. *F. psychrophilum* is considered one of the most important salmonid pathogens worldwide because of the large number of fish deaths caused by infection and the resulting economic impact among commercial aquaculture producers.

In 2003, NCCCWA molecular biologist Greg Wiens and microbiologist Tim Welch began talking to industry trout producers about their issues and concerns, and cold-water disease came up most often. "We worked closely with industry partner Scott LaPatra at Clear Springs Foods in Idaho, and obtained *F. psychrophilum* strains from his facility," says Wiens. "We sequenced the genome of one strain and used this information to design PCR [polymerase chain reaction] assays to measure genome variation. Now we can better distinguish between isolates, and we are trying to determine the impact these different variants have on trout disease resistance."

Fish can be infected simultaneously with multiple strains—up to three pathogen strains were found in a single fish. Studies

CINDY LEDBETTER (D1937-1)

Catfish infected with Ich, caused by *Ichthyophthirius multifiliis,* a protozoan parasite that is responsible for the white spots on the fish.

are under way to test the responses of fish when exposed to *F. psychrophilum* variants.

To mitigate the pathogen's impact, Wiens has been working since 2005 to selectively breed rainbow trout for improved resistance to bacterial cold-water disease. "It is a large project involving many scientists and personnel at our facility," including geneticist Tim Leeds, computational biologist Roger Vallejo, and Jeff Silverstein, who is now the national program leader for ARS's national program on aquaculture.

"After two generations of selection, we found there was a 45-percent improvement in survivability of the resistant trout," says Wiens. "Small-scale field trials are currently under way in Utah and Idaho to determine how well our fish perform on farms when they are subjected to natural challenge with the pathogen. A larger field trial is planned for 2011." These resistant lines will offer breeders a genetic resource for improving disease resistance in commercial populations.

Wiens and his colleagues also noticed something quite interesting about the resistant fish: They have larger spleens. The researchers have now found that spleen size is highly heritable and that there is a genetic correlation with bacterial cold-water disease resistance. Understanding the relationship between resistance and spleen size may provide clues to help researchers select for resistance more rapidly and may reveal important insights about the immune response generally. They are now trying to map the rainbow trout genes affecting disease resistance and variation in spleen size. These genetic studies are being conducted by geneticist Yniv Palti and center director Caird Rexroad III.

"Ichy" Fish

Dave Straus in Stuttgart, Arkansas, is researching treatments to control disease on catfish, specifically Ich and fungi. "Ich" is short for *Ichthyophthirius multifiliis,* a protozoan parasite that at maturity appears as white spots on infected fish—the scourge of anyone who has owned an aquarium. Because catfish don't have scales, they are particularly vulnerable to this parasite.

"Ich is considered the most prevalent parasite worldwide in ornamental fish, baitfish, and food fish," says Straus, an aquatic toxicologist at the Harry K. Dupree Stuttgart National Aquaculture Research Center. "It is less common in U.S. aquaculture because of management techniques, but when it occurs, it can kill all the fish in a pond or raceway. Based on results of a 2003 survey, it was calculated that Ich was directly responsible for $1.2 million in losses to the catfish industry."

The freshwater fungus *Saprolegnia* is another major pathogen in fish culture, killing eggs and invading wounds and lesions on juvenile and adult fish.

One effective treatment for Ich on fish and fungus on eggs is copper sulfate. Straus's lab conducted research to determine effective doses for each use. "Copper sulfate is the only practical treatment to control Ich in catfish ponds that average about 10 acres in area. It is easy to use, effective, inexpensive, and safe to the person using it," says Straus. "Approved treatments for fungus on eggs—formalin and hydrogen peroxide—are much more expensive; both compounds are hazardous, and there are human safety concerns as well as storage precautions."

During the life cycle of Ich, only the free-swimming stage is susceptible to treatments. Once Ich attaches to the fish, it burrows under the skin, and copper sulfate treatments are rendered ineffective.

"Copper sulfate is not approved by the U.S. Food and Drug Administration (FDA) for therapeutic use in aquaculture, but regulatory action has been deferred pending the outcome of ongoing research at our lab," says Straus. "Copper sulfate is approved by the U.S. Environmental Protection Agency as an algicide and molluscicide. Fish farmers use copper sulfate to control cyanobacteria that cause off-flavor in fish and to control snails that transmit parasitic flatworms to fish."

Straus is working with corporate sponsor Freeport-McMoRan Copper and Gold of Phoenix, Arizona, to obtain FDA approval to use copper sulfate as a treatment for Ich in earthen production ponds and as a fungicide on eggs in catfish hatcheries. Research has recently been completed for all technical sections for both label claims—control of Ich on catfish and fungus on catfish eggs.

"All technical sections are complete for the approval for Ich in catfish except for the environmental safety. We plan to resubmit the environmental assessment to the FDA by the end of the year with the additional information the agency requested," says Straus.

There's a "Vacc" for That

Advances in research, including new fish vaccines developed to tackle pathogens such as *Streptococcus iniae* and *S. algalactiae,*

DAVE STRAUS (D1935-1)

Healthy catfish eggs treated with copper sulfate.

DAVE STRAUS (D1936-1)

Fungus on catfish eggs not treated with copper sulfate.

STEPHEN AUSMUS (D1162-12)

Fish lab manager James Everson (left) and ARS geneticist Yniv Palti collect rainbow trout fingerlings to evaluate growth rate and resistance to disease.

STEPHEN AUSMUS (D1944-19)

ARS molecular biologist Greg Wiens (left) and ARS microbiologist Tim Welch examine a culture of *Flavobacterium psychrophilum,* a bacterial pathogen of rainbow trout.

could help protect America's aquaculture industry when it needs it most.

Scientists at the ARS Aquatic Animal Health Research Unit in Auburn, Alabama, and its worksite laboratory in Chestertown, Maryland, are developing vaccines to protect farm-raised catfish and nearly two dozen other aquatic animal species that are vulnerable, says microbiologist Phillip H. Klesius.

Klesius, research leader at Auburn, working with colleagues Joyce J. Evans in Chestertown and Craig Shoemaker and Julia Pridgeon in Auburn, has been recognized with several professional awards for innovative work over the past decade. Their successful vaccine against the pathogen causing enteric septicemia of catfish has been widely adopted across the catfish industry.

"We have been producing vaccines using modified live bacteria that are no longer viable pathogens," Klesius says. "We've been able to patent several vaccines in the last 10 years and then license them to private firms."

Researchers have made the process of modifying the genetic makeup of pathogens to make them nonvirulent seem almost routine. They are now able to develop vaccines that expose fish or other animals to low doses of modified forms of the pathogen, which allows the fish to build up a lifelong immunity. "We use a live, modified vaccine that has enough similarity with the pathogen to create immunity," says Klesius.

Streptococcus presents a serious threat to the industry. The pathogen is ubiquitous and can occur in wild fish and in food fish. It can be found on almost any size farm. Pridgeon and Klesius have developed a modified live *S. iniae* vaccine that appears to be superior to inactivated or killed vaccines.

A technique in which fish are immersed in water containing the modified pathogen has been used to vaccinate large numbers of fish, but current work on developing alternative methods of vaccine delivery through feeding are promising.

Through field trials conducted in 1996 in 10 ponds on a commercial farm, David Wise, a research professor at Mississippi State University, and retired scientist Kurt Schuster found that the vaccine for enteric septicemia of catfish increased the survival rate of the fish by more than 12 percent. Their study also showed that producers using the vaccine could expect returns to increase by $1,800 per acre.

Klesius, Evans, Pridgeon, and Shoemaker are working with an eight-member research team to determine genetic variation in response to vaccines and pathogens, study disease progression in fish infected with multiple pathogens, develop more sophisticated ways to detect pathogens, describe how immunity—innate and acquired—works in aquatic animals and find opportunities to enhance it.—By **Sharon Durham** and **Chris Guy,** ARS.

This research supports the USDA priorities of ensuring food safety and promoting international food security and is part of Aquaculture, an ARS national program (#106) described at www.nps.ars.usda.gov.

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EAT FISH! Nutrition Studies Zero In on Health


FLORIDA DEPARTMENT OF AGRICULTURE & CONSUMER SERVICES

Seafood can be a source of DHA and EPA, omega-3 fatty acids that are essential for good health. ARS scientists are determining more about the health-promoting roles of these compounds.

Like salmon?

Find that anchovies are one of your "must have" pizza toppings?

If you're a fan of such fish, your body is probably thanking you right now.

These and some other fish, including mackerel, herring, albacore tuna, and more, are rich in healthful natural compounds known as "omega-3 fatty acids." Ongoing studies by chemist Darshan S. Kelley and his Agricultural Research Service and university coinvestigators are helping uncover new details about how these fish-oil components help protect us from chronic diseases.

Kelley is based at the ARS Western Human Nutrition Research Center at the University of California-Davis.

Trio of Fatty Acids Studied

In an early study with laboratory mice, Kelley and colleagues took a closer look at the interplay of three different fatty acids, commonly referred to by their abbreviations: CLA, DHA, and EPA.

The type of CLA, or conjugated linoleic acid, that the team investigated is known as "trans-10, cis-12 CLA" and is found in partially hydrogenated vegetable oils and some dietary supplements. Its interactions with DHA and EPA, from fish oils, are of interest to nutrition scientists in the United States and abroad.

Why the interest in CLA, DHA, and EPA?

Results from a handful of studies, conducted by researchers elsewhere, indicate that feeding fish-oil supplements—in conjunction with CLA supplements—to lab animals prevents certain harmful side effects attributed to CLA. Those side effects include insulin resistance and fatty liver.

Those studies, however, have not conclusively determined whether it was fish oils' DHA, EPA, or both that provided the protection.

DHA (docosahexaenoic acid), and EPA (eicosapentaenoic acid) occur in different amounts and ratios in fatty or oily fish like those mentioned earlier, as well as in some mollusks, crustaceans, and other seafood; and, of course, in fish-oil supplements.

Insulin Resistance and Fatty Liver Monitored

The insulin resistance that Kelley and colleagues monitored is a condition in which the body isn't able to efficiently use its own insulin, a hormone, to remove glucose (sugar) from the bloodstream. If left untreated, insulin resistance can lead to diabetes and harmful buildup of glucose in the bloodstream.

An estimated 26 million to 57 million Americans are insulin resistant.

The liver condition that the team tracked is known as "non-alcoholic fatty liver disease." It results from accumulation of excess fat in the liver. Causes include diabetes and obesity. Afflicted liver tissue may harden and scar, sometimes resulting in cirrhosis of the liver or liver cancer.

Kelley's 8-week test with 50 laboratory mice indicates that DHA protected against both CLA-induced insulin resistance and

CLA-induced non-alcoholic fatty-liver disease. In contrast, EPA offered partial protection against CLA-induced fatty liver disease and no protection against insulin resistance.

Four Different Rations Used

The mice were fed one of four different kinds of rations: one that contained no CLA, DHA, or EPA; another with CLA amounting to 0.5 percent of the total weight of the feed; a third feed with CLA as 0.5 percent plus 1.5 percent DHA; or, in a fourth ration, 0.5 percent CLA plus 1.5 percent EPA.

"We chose these amounts of DHA and EPA because they are in line with amounts in the fish oils that were used in several previous animal studies," says Kelley. "That gave us a better basis for comparison."

Kelley published the study findings in a 2007 issue of *Metabolic Syndrome and Related Disorders* with colleagues Madhuri Vemuri, the senior author and former graduate student in Kelley's lab; Giovanni Bartolini of the Davis team; and Reuven Rasooly and Bruce E. Mackey, with ARS in Albany, California.

The study was the first to demonstrate that DHA provided protection against CLA-induced insulin resistance and non-alcoholic fatty liver disease.

Now, as a followup to the mouse investigation, Kelley would like to work with adult human volunteers who are prediabetic.

The intent?

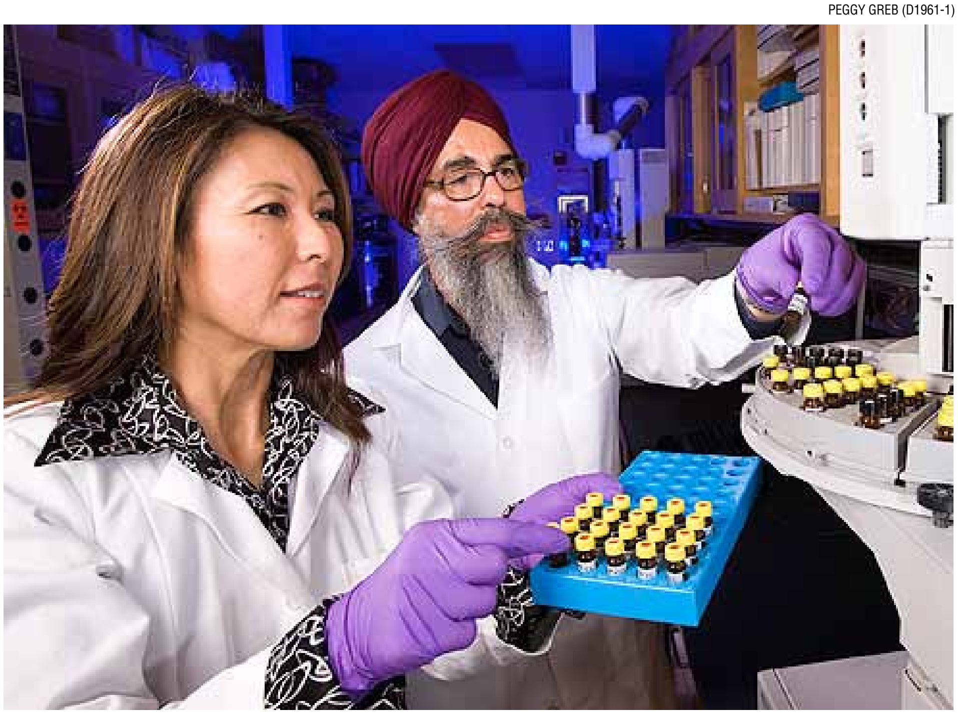
To determine whether DHA can improve the volunteers' ability to use insulin efficiently and thus help delay onset of diabetes.

"We're particularly interested in the mechanisms that DHA and EPA use and the sites upon which they act," Kelley says. "We're also interested in the genes that control these mechanisms." This information could help shape larger studies and, eventually, may lead to new dietary recommendations.

Adiponectin: A Hormone From Fat Cells

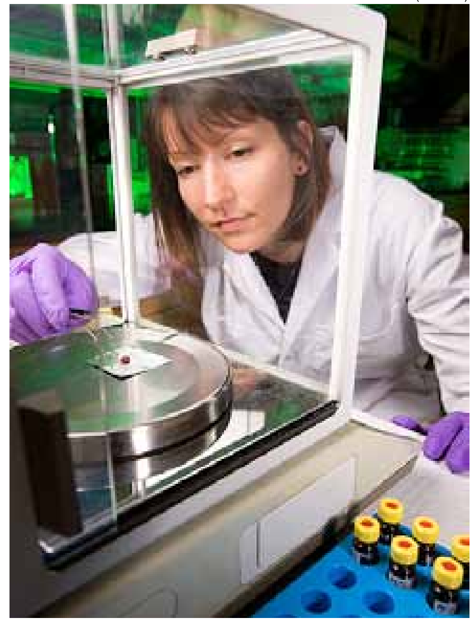
One mechanism of interest is DHA's interaction with a recently discovered hormone known as "adiponectin." Scientists already know some of the basics: Adiponectin is produced by fat cells in adipose tissue; low levels of adiponectin have been associated with insulin resistance; CLA depletes the adipose tissue in which adiponectin is made; and DHA can restore adipose tissue, thus indirectly increasing adiponectin levels.

PEGGY GREB (D1961-1)



Molecular biologist Yuriko Adkins (left) and chemist Darshan Kelley load lipid samples extracted from tissue for analysis of fatty acids by gas chromatography/mass spectroscopy.

PEGGY GREB (D1962-1)



Graduate student Dawn Fedor weighs liver tissue before analyzing it for lipid content.

Kelley and coinvestigators noted that laboratory mice fed the CLA-plus-DHA feed had higher levels of adiponectin than did the mice that were given the feed that contained CLA but not DHA. Thus, "increasing adiponectin levels may be an important mechanism by which DHA protects against insulin resistance," says Kelley. "But we need to know more."

According to Kelley, several dozen studies with human volunteers have been conducted in the past decade to determine the effect of EPA and DHA mixtures—from fish or fish-oil supplements—on insulin resistance. With coresearcher Dawn Fedor, a University of California-Davis graduate student, Kelley reviewed 3 years' worth of these investigations in an article published in 2009 in *Current Opinions in Clinical Nutrition and Metabolic Care.*

In general, and for many different reasons, study results are inconsistent. The review underscores the need for new investigations, with larger numbers of volunteers, to more clearly define the precise relation of DHA and EPA to insulin resistance.—By **Marcia Wood,** ARS.

This research is part of Human Nutrition, an ARS national program (#107) described at www.nps.ars.usda.gov.

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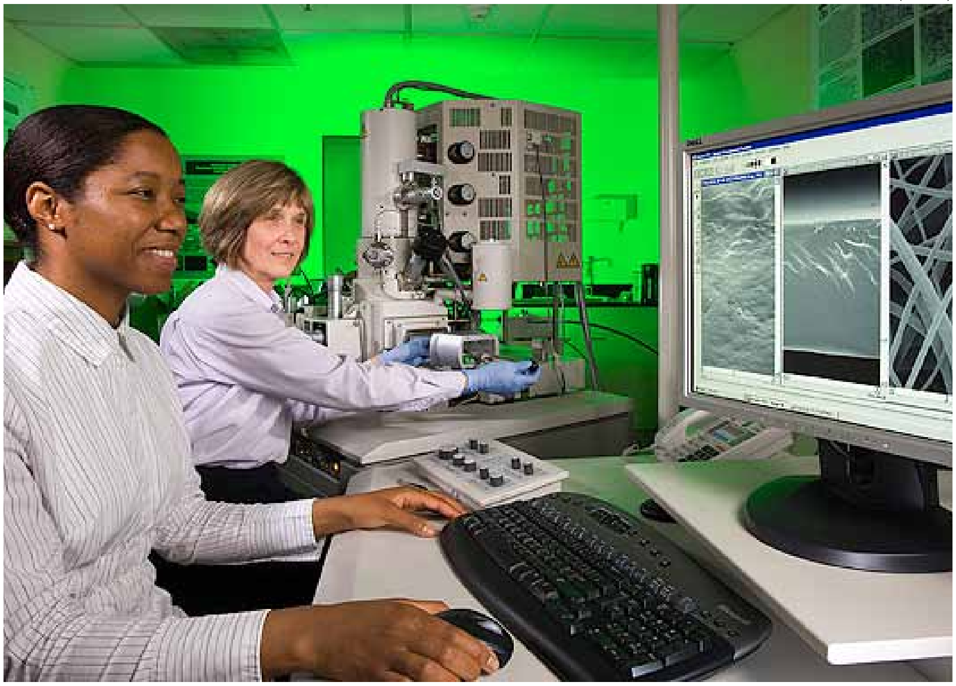
FISH LEFTOVERS: New Life Ahead for

PEGGY GREB (D1951-1)



Technician Tina Williams (left) and botanist Delilah Wood examine scanning electron microscopy images of electrospun nanofibers containing fish gelatin.

Food technologists Cindy Bower and Peter Bechtel spend countless hours up to their elbows in buckets full of aquatic spare parts—fish heads, tails, bones, livers, and more. It's all known as "fish byproducts," the leftovers after premium fillets and other edible sections of wild-caught fish are removed and sold.

Harvested from cold waters of the Pacific Ocean, these fish are cleaned and packed in large processing plants in Alaska. Some of those plants are located on the Kodiak Island outpost that serves as the new headquarters for the Agricultural Research Service's subarctic aquaculture research.

More than half of all wild-caught fish harvested and processed in the United States for human consumption—about 2 million tons—comes from Alaska. But it's the 1 million tons or so of undervalued bits and pieces of salmon, pollock, cod, and other marine fish that interest Bechtel and Bower.

"Most of the leftovers are ground, dried, and then sold as ingredients for products such as aquaculture feeds or pet foods," explains Bechtel. "But these leftovers could be directed to higher value uses. That could increase the potential value of Alaska's fishing industry byproducts by greater than $100 million," he estimates.

"Our job is to find ways to increase the value of the byproducts," says Bower.

The researchers have already conducted an array of projects designed to do exactly that, says Bower, who joined lead scientist Bechtel about 6 years ago, when they were based in Fairbanks. Many of the studies were conducted in partnership with scientists from the University of Alaska-Fairbanks as well as with researchers in more than a half dozen other states.

Low Tech, But High Value

One way Bower hopes to make fish discards more marketable is through smoke-processing. Pink salmon fish heads are rich in oil. "Smoking the fish heads preserves the quality of the oil by delaying oxidation. The process also boosts the antioxidant content of the oil and gives it an appealing, smoky flavor," she says.

Bower and colleagues described their smoking research in a 2009 article in the *Journal of Food Science.*

What's more, she has established a cooperative agreement with scientists at the University of Maine-Orono to explore the possibility of using the aroma-rich oil in foods such as gourmet cheeses.

In other work, Bower and coinvestigators have developed a low-cost, low-tech method for efficiently drying skins of Alaskan pollock. Drying makes the skins lighter and significantly less expensive to ship out of state to factories where they can be used to make gelatin. There is a growing market for fish gelatin among people who don't eat products made from either pigs or cows—the major sources of gelatin. At present, Alaska doesn't have any gelatin-processing facilities.

The process that Bower and colleagues developed relies on drying agents, also referred to as "desiccants," rather than hot air. The desiccants are commonplace, regenerable, and already approved for food use. In tests with pollock skins, the scientists determined

PEGGY GREB (D1949-1)

Chemist Bor-Sen Chiou examines a film made from nanofibers after electrospinning on aluminum foil.

the relative efficiency of drying skins for 24 hours using any of four different drying agents. The findings appeared this year in the *Journal of Food Science.*

In Hawaii, Fish Say "Aloha" to New Feeds

At the Oceanic Institute in Waimanalo, Hawaii, scientists are collaborating with Bechtel in testing feeds made from Alaska's fish-processing leftovers. The Hawaii scientists are formulating new and improved feeds for succulent Pacific white shrimp and for Pacific threadfin or "moi," a delicious white-fleshed species once reserved exclusively for Hawaii's royalty.

Funded by ARS, scientist Dong-Fang Deng and her colleagues at Waimanalo fashion the fish leftovers into feed pellets. They then characterize the nutrient composition of the feeds, evaluate their ability to attract the shrimp and moi, estimate the feed's digestibility, and assess the growth of the animals.

Recent tests have shown that many of the Alaska fish parts act as feeding stimulants that, as their name implies, stimulate fish to eat their rations. That occurred in studies with Pacific white shrimp that were fed plant-protein-based feeds to which fish-processing leftovers had been added.

In an earlier ARS-funded study with moi, researcher Ian Forster, formerly at the Oceanic Institute and now at the Canadian Department of Fisheries and Oceans, found that the nutritional quality of feeds made with discarded portions of Alaskan pollock and cod was equivalent to that of feed made from Norwegian fishmeal, generally regarded as the highest standard in the aquaculture feed industry.

An article by Forster and coinvestigators, published in the *Journal of the World Aquaculture Society,* has details.

In another early investigation—this one with Pacific white shrimp—Forster and colleagues showed that the nutritional quality of feed made with Alaska fish byproducts, mainly pollock, was also comparable to that of Norwegian fishmeal. Their article in the *Journal of Aquatic Food Product Technology* describes the study.

With further work, Alaska's fish-processing leftovers may find a niche in Hawaiian aquaculture. Says Deng, "We're looking at how they fit into our overall objective, which is to develop optimal feeds that are nutritionally balanced, cost effective, and environmentally friendly."

Fish Gelatin: Ultra-High-Tech Biomedical Uses Ahead?

There's also a California connection to the Alaska research. Gelatin from the discarded skins of Alaskan pollock may someday be put to intriguing new medical uses, according to ARS chemist Bor-Sen Chiou. He's developing strong yet pliable sheets, called "films," made from a blend of the gelatin and a bioplastic called "polylactic acid," or PLA, from fermented corn sugar.

Chiou is doing the work in conjunction with colleagues in California, where he is based, and with the Alaska team.

PEGGY GREB (D1950-1)

Food engineer Roberto Avena-Bustillos pours fish gelatin solution into a pan for drying. The fish gelatin was extracted from the skins of Alaskan pollock.

The fish- and corn-derived films might in the future be used to produce semi-synthetic tissue to speed repair of injured bone or cartilage, for instance.

Chiou is with the ARS Western Regional Research Center in Albany—near San Francisco. There, in an ultra-high-tech process known as "electrospinning," the fish gelatin and the PLA are spun together to form slender, submicroscopic fibers. When amassed, these nanofibers form sheets of milky-white film.

At tomorrow's tissue-engineering labs, the films could be "seeded" with cultures of human cells. The nanofibers would provide a matrix on which the cells could replicate. Later, the tissue resulting from the replicating cells would be used as transplants.

The natural materials that are the basis of the nanofibers—fish and corn—are not expected to pose problems such as allergic reactions. In fact, some of today's surgically implanted medical devices are made of PLA or contain components made of it.

Chiou, his Alaska colleagues, and his California associates—food engineer Roberto Avena-Bustillos of the University of California-Davis and Albany technicians Haani Jafri and Tina Williams—may be the first to use a blend of fish gelatin and corn-derived plastic to make next-generation nanofibers. It's a fascinating application of harvests from both land and sea.—By **Chris Guy**, **Stephanie Yao**, and **Marcia Wood,** ARS.

This research is part of Aquaculture (#106) and Quality and Utilization of Agricultural Products (#306), two ARS national programs described at www.nps.ars.usda.gov.

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IN-DEMAND FISH: Making Sure They're

Popular fish like salmon, catfish, and tilapia are coming under the close scrutiny of Agricultural Research Service food-safety scientists Andy Hwang and Kathleen Rajkowski. They're discovering more about how to prevent foodborne pathogens from contaminating these and other delicious, good-for-you seafood. Both scientists are based at the ARS Eastern Regional Research Center in Wyndmoor, Pennsylvania.

Hwang, a food technologist, has completed a series of studies in which he's simulated—in his laboratory—commercial processes used today for preparing smoked salmon. A gourmet treat, smoked salmon is typically sold in vacuum packages that have a refrigerator shelf life of about 3 to 8 weeks, according to Hwang. Trouble is, pathogenic microbes like *Listeria monocytogenes* can live at refrigerated temperatures, so it's important to get rid of these harmful microbes before the product leaves the processing plant.

Smoked salmon, pricey and, when properly prepared, delicate in texture, is often served in thin slices with bagels and cream cheese or as an appetizer, stacked on toast-type crackers with red onion and a splash of lemon juice. Too, some sushi bars feature smoked salmon surrounded by sticky rice and snugly wrapped in seaweed.

Hwang is looking for ways that processors can protect the pleasing flavor and texture of smoked salmon while reducing or eliminating contamination by *L. monocytogenes* or other foodborne pathogens.

PEGGY GREB (D1966-1)

Food technologist Andy Hwang and technician Stacy Raleigh study the effect of smoking temperature on survival of *Listeria monocytogenes* on smoked salmon.

At the Smokehouse

Smoked salmon is typically prepared by using what's known as "wet brining" or "dry brining" to cure the raw fillets before smoking. Fish cured with a wet brine are soaked in a solution of water, salt, and sugar, which preserves the fish, helps it retain moisture, and enhances its flavor. The brine may also include spices or liquid smoke, like the kind home chefs use for a backyard barbeque.

With dry brining, the salt and other compounds are rubbed on the fillets and later rinsed off before the fish is smoked.

The smoking process takes place in special smoking ovens in which wood chips are burned to smoke the cured fillets. Most processors opt for cold smoking, which uses temperatures of 68˚F to 86˚F to smoke—but not cook—the fillets. Cold-smoking takes about 3 to 4 days.

Hot-smoking, a lesser-used option for salmon, uses temperatures of about 140˚F and takes about 6 to 10 hours. Hot-smoking cooks the fish, giving it a different taste and texture than cold-smoked fish.

Always Safe To Eat

Many Combinations Tested

In a series of experiments, Hwang and colleagues Shiowshuh Sheen and Vijay Juneja at Wyndmoor exposed cooked salmon samples, prepared with various concentrations of salt and smoke compound (from burning wood chips or liquid smoke), to midrange temperatures—between 104˚F and 131˚F. "The temperatures were higher than those used for cold-smoking but not quite as warm as hot-smoking," explains Hwang. "We wanted to provide a range of alternative smoking temperatures for processors to consider and to show them the level of *Listeria* inactivation they might be able to achieve at various temperatures and various combinations of salt and smoke compound."

The scientists cooked the fillets for the tests to kill any existing microbes before inoculating the fish with *Listeria*. Not surprisingly, smoking temperature was the single most important factor for inactivating the microbe. "Every 9˚F increase in temperature resulted in a 10-fold increase in rates of inactivation of the *Listeria,*" Hwang reports.

The researchers used data from the study to create a new, first-of-its-kind formula, or mathematical model, for food processors and their food-safety consultants to use in choosing the optimal combination of temperature and concentrations of salt and smoke compound.

"Users can plug into the model the salt concentration, smoke-compound concentration, and smoking temperature of their choice to predict what effect this combination may have on *Listeria* levels," says Hwang. "Salt and smoke-compound concentrations and smoking temperature affect taste, texture, and other key qualities of the smoked fish, so processors often have their own unique combination of these three factors. We constructed the model to accommodate a wide range of choices."

The team's 2009 article in the *Journal of Food Science* has details.

Now, Hwang intends to test these laboratory findings at a smokehouse and monitor the safety of the smoked salmon as it makes its way through the distribution chain, from wholesaler to retailer to restaurant or home.

And as a followup to a preliminary study that he and Sheen described in another 2009 *Journal of Food Science* article, Hwang wants to discover more about the extent to which other microbes—benign or harmful—can colonize the fillets and help or hinder *Listeria's* survival.

Powerful Tactics That Don't Require Heat

Meanwhile, colleague Rajkowski, a food microbiologist, is determining how to prevent certain foodborne pathogens from contaminating fish fillets. She's using tilapia and catfish fillets for this research. "Even though foodborne illnesses are not commonly associated with either of these fish," says Rajkowski, "we chose them for our research because they are the two most commonly consumed kinds of fish fillets in the United States today."

Microbes that she's studying include not only *Listeria* but also *Salmonella, Shigella, Staphylococcus, Pseudomonas*, and *Escherichia coli* O157:H7.

In one study, Rajkowski is determining the correct cooking times and temperatures for packaged tilapia fillets. Instructions for cooking fillets are sometimes based on visual determination—what the fish looks like.

"Instructions might require you to know what the fillet looks like when it 'flakes easily with a fork,'" she says. "Not everyone knows what's meant by that. We want to provide science-based cooking instructions that are precise and easier for everyone, even beginning cooks, to follow."

Rajkowski is continuing research on heat-free ways to reduce levels of harmful microbes. Overheating can easily ruin the taste and texture of fish.

In an early experiment with both frozen and thawed tilapia and catfish fillets, Rajkowski artificially inoculated the fillets with *L. monocytogenes* and then determined the amount of ionizing radiation needed to reduce the pathogen's population by 90 percent. The dosages required to achieve that level of safety were nearly the same for both kinds of fish, Rajkowski found. Published in the *Journal of Food Protection* in 2008, the study was the first to identify the dosages needed to effectively reduce *Listeria* in these popular fish products. Her results were similar to those that reduce *Listeria* in ground beef.

PEGGY GREB (D1964-1)

Food microbiologist Kathleen Rajkowski places a frozen catfish fillet into a device for surface decontamination by pulse UV treatment.

Rajkowski is also testing the effects of ultraviolet (UV) light in combating another pathogen, *Shigella sonnei*. Like *Listeria, Shigella* can cause gastrointestinal illness. For one investigation, Rajkowski applied a solution of *S. sonnei* to the surface of frozen tilapia and then exposed the samples to UV light. The treatment resulted in a 99-percent reduction of the pathogen. In contrast, tests with small samples of fresh tilapia showed that the UV treatment did *not* kill the pathogen. But exposing the fillets to pulsating beams of high-intensity UV light reduced the pathogen by 99 percent. Rajkowski documented the study in 2007 in *Ice World Journal.*

Fish that Hwang and Rajkowski are investigating are a good, low-fat source of high-quality protein. That's reason enough to make sure these fish, and others from farm and sea, remain pathogen-free and safe for us to eat.—By **Marcia Wood,** ARS.

This research supports the USDA priority of ensuring food safety and is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

Cheng-An (Andy) Hwang and Kathleen Rajkowski are with the USDA-ARS Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038; (215) 233-6416 (Hwang), (215) 233-6440 (Rajkowski), andy.hwang@ars.usda.gov, kathleen.rajkowsi@ars.usda.gov. ✻

ARS National Research Program for Aquaculture

Fish and shellfish farmers are facing formidable challenges. To meet today's growing demand for seafood in a sustainable way, U.S. aquaculture producers, especially small and mid-sized ones, need new ways to cut their production costs while improving product quality and reducing environmental impacts.

ARS's strong national research program in Aquaculture (#106) is bringing resources to bear to coordinate genomics, genetics, nutrition, health, and physiology in research projects to enhance the farming of fish and other aquatic species. National program #106 also cooperates with ARS research programs in the natural resources and sustainable agriculture areas as well as with Food Safety (national program #108) and Quality and Utilization of Agricultural Products (national program #306).

One of the priorities of the ARS aquaculture program is to develop genomics libraries and bioinformatic tools for current and emerging farmed aquatic species and incorporate that information into breeding research to enhance available germplasm. Data from breeding, nutrition, and health studies feeds back into genomics work and provides direct results to enhance production.

ARS breeding programs, several of which are run in conjunction with university counterparts, are developing fast-growing fish and shellfish with enhanced disease resistance, improved fillet yield, and better reproduction. These breeding programs are based on traditional selective breeding practices, though they are moving quickly to incorporate information from genomics research into the selective breeding programs.

As ARS expands the genomics information available for farmed aquatic species, breeding research is able to incorporate genomic information and improve the selection process. ARS has already identified individual genes for growth, metabolism, muscle development, and fish health.

Genomic data is now enabling researchers to develop multitrait selection projects, using tools such as genetic markers for important genomic locations. Development of other tools, such as new methods for cryopreserving aquatic germplasm, is helping to enhance research and provide an important backup for aquatic species. Long-term cryopreservation storage of gametes for other aquacultured species, such as striped bass, tilapia, shrimp, and oyster, is being developed.

ARS scientists are also working on methods to genetically identify and reproductively isolate domesticated stocks to prevent unintended interactions between farmed and wild populations. ✻

Worth a Look: The National Agricultural Library's Aquaculture Web Pages

Whether you're interested in the business of farming fish, the science of aquaculture, or simply want to create a fish pond for your home garden, you'll want to browse the aquaculture web pages developed and hosted by the Agricultural Research Service's National Agricultural Library in Beltsville, Maryland, and Washington, D.C. (**tinyurl.com/NALaqua**).

Part of the library's Alternative Farming Systems Information Center, this fish-focused venue offers links to more than 160 carefully selected websites. Browse the home page for specially featured sources that open the door to information on everything from farming catfish, carp, salmon, shrimp, and many more species to business planning for a fish-farming operation.

Some of these featured links connect you to National Sea Grant sources on environmentally friendly fish farming and to time-saving searches on many aspects of aquaculture (**tinyurl.com/aquasearch**). These comprehensive searches include the National Agricultural Library's own AGRICOLA (**AGRIC**ultural **O**n-**L**ine **A**ccess) database, which encompasses the library's substantial collection. Holdings include articles from major scientific and industry journals and more than 4,000 books—old and new—on an impressive array of aquaculture subjects.

The collection's oldest aquaculture volume might well be a 1786 compendium from Britain that advises "country gentlemen" on the basics of establishing and maintaining rural fish ponds "for pleasure and profit." Newer acquisitions include the Mississippi-based Southern Regional Aquaculture Center's 2010 book on fish genetics.

Other links take you to, for example, research organizations, federal agencies, databases of laws and regulations, and sources of graphics and images suitable for use by students and professionals alike.

Says program support assistant and aquaculture site content contributor Rebecca Thompson, "Anyone interested in aquaculture is likely to find something new and interesting on our web pages."—By **Marcia Wood,** ARS. ✻

DENNIS ROE (D1947-1)

Winter Canola

An All-Around Winner

One day last fall, Agricultural Research Service (ARS) agronomist Frank Young began to fuel up a school bus with a biodiesel blend made with winter canola. The school kids who were watching him clapped and cheered when Young told them that the biodiesel would make the bus smell like French fries.

Winter canola being harvested in north-central Washington.

The students—all members of the Colville Confederated Tribes—aren't the only ones who've been applauding Young, who works at the ARS Land Management and Water Conservation Research Unit in Pullman, Washington. He's been heading up a long-term study that suggests winter canola production in some parts of Washington State could have something for everyone: a weed-suppressing alternative crop for farmers, feed supplements for cattle, biodiesel for school buses, a new income stream for members of the Colville Confederated Tribes—and a little local color.

"Winter canola blooms in May," Young says. "And when there's a whole field of yellow flowers, everyone wants to know what it is."

Looking for Alternatives

Pacific Northwest farmers who produce winter wheat must also find ways to control germination and growth of winter annual grass weeds, such as jointed goatgrass, feral rye, and downy brome, in their crop fields. When the seeds from these grasses get harvested with the wheat, they reduce the quality of the crop, which results in lower profits for the harvested wheat.

But finding a suitable economical alternative crop that competes with the annual grasses has been a challenge. For instance, growers in this region have been reluctant to plant winter canola crops because the seedlings struggle to emerge and survive in the dry soils. Plants that do live until winter arrives are then susceptible to freezing because they don't have enough snow cover for protection.

Young's work is part of a larger partnership for north-central Washington that includes ARS, the Colville Confederated Tribes, Washington State University Extension, and local farmers and schools. The partnership was formed to develop alternative crops for providing food, fuel, and new jobs for the region. And it supports efforts by the U.S. Department of Agriculture (USDA) to reduce dependence on foreign oil and create a new industry that benefits farmers and rural communities.

Young began his research on winter canola with Dennis Roe, a USDA Natural Resources Conservation Service agronomist who was already working with members of the Pacific Northwest Indian Coalition on growing canola as a biodiesel crop. A few members of the Colville Confederated Tribes had let Roe set up several spring canola research plots on their property in Omak, Washington—and all they had to do was water them and watch them flourish.

Encouraged by the oilseed yields from the trial spring canola crops—a little less than 1,000 pounds per acre—the scientists decided to see whether they could make the production of winter canola even more feasible than the production of spring canola. So they began a long-term study that evaluated winter canola production in Okanogan County in north-central Washington, where rainfall averages 9 inches and winters have adequate snow cover to protect the young plants.

A biodiesel blend containing oil from winter canola is pumped into a Paschal Sherman Indian School bus. Pictured left to right: Washington State University extension specialist Phil Linden, Colville Confederated Tribes member Ernie Clark, ARS agronomist Frank Young, ARS technician Larry McGrew, and local grower Ed Townsend.

CARLA DES VOIGNE (D1948-1)

The researchers varied the planting dates, planting rates, and other establishment techniques and found that when seeds were planted in early September, the emerging seedlings did not have enough time to bulk up before the onset of winter. But they obtained consistently good yields—an average of 1,300 pounds per acre—when they planted in mid-August on 28-inch row spacing.

As a result of this research, wheat farmers in Okanogan County have begun to plant winter canola to rid their fields of feral rye and diversify their market options. And the canola crop may also help the soil store water—something winter wheat stubble could not do well. "Winter wheat has hairy, fibrous roots that don't penetrate far below the soil surface," Young says. "Winter canola has roots with a huge taproot, like a carrot. It grows 5 to 6 feet deep, and we think it creates a channel through the soil that allows more water to infiltrate, which subsequent crops can use."

The research has also been used by the USDA Risk Management Agency to expand its program for insuring canola against crop loss to Douglas and Okanogan counties. For the first time, this allows growers in these counties to obtain crop insurance for canola, which helps reduce their risks when drought or other natural weather disasters strike.

The USDA-ARS Land Management and Water Conservation Research Unit contributes to the USDA Northwestern Regional Biomass Research Center, which is one of five national centers whose mission is to help accelerate the establishment of commercial biomass production from farms and forests in ways that do not disrupt food, feed, and fiber markets and that enhance natural resources quality.

But Wait—There's More!

The payoffs from Young's research don't stop in the field. Many of the farmers in this region lease crop land from the Confederated Tribes of the Colville Reservation, and the Colville tribe plans to purchase the canola seed from the growers and process it in a biodiesel production facility that they own.

The tribe plans to sell crushed canola meal back to the farmers as a protein feed supplement for cattle during the winter. They also plan to use biodiesel they produce from the canola seed oil to power the tribes' fleet of 100 logging trucks and other agency vehicles as well as their 15 school buses.

"When the buses run on an 80/20 petroleum diesel/canola biodiesel blend, it's enough to eliminate black soot from the exhaust," Roe says.

In the future, as much as 20,000 acres of winter canola could be grown on Colville tribal lands. This acreage could support production of enough oilseeds for 2 million gallons of canola-based biodiesel and 6,500 tons of high-protein canola meal every year. These activities have the potential for generating annual gross revenue of $8.8 million for the tribe and the surrounding community.

"Everyone wants this to succeed," Roe says.

Young is delighted with how the research has provided Washington growers with a new crop—and more money. "One grower went from planting winter canola on 15 acres in 2007 to planting it on 240 acres in 2009, and his profits from winter canola ended up beating his profits from winter wheat," he says. "This has been an incredibly exciting and rewarding project."—By **Ann Perry,** ARS.

This research is part of Soil Resource Management (#202), Agricultural System Competitiveness and Sustainability (#216), and Bioenergy (#213), three ARS national programs described at www.nps.ars.usda.gov.

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Invention May Help Improve School Lunch

National School Lunch Week is being held this month, and Agricultural Research Service (ARS) researchers have provided another reason to celebrate—a new technology that holds promise to make students' mealtimes safer and more appealing.

It all started several years ago when school food-service personnel noticed that there were too many bone fragments in poultry used to make meals for the National School Lunch Program. The National School Lunch Program is a federally assisted meal program operating in public and nonprofit private schools and residential child-care institutions. About 31 million children participate in the program each weekday during the school year.

Complaints from school food-service providers led to researchers at the ARS Environmental Microbial and Food Safety Laboratory (EMFSL), in Beltsville, Maryland, being asked to come up with a method to help meat producers detect bone fragments. "The meat comes from processing plants and is made into little cubes for use in dishes such as chicken nuggets," says Alan Lefcourt, a biomedical engineer with the laboratory.

EMFSL physicist Moon Kim, agricultural engineer Yud-Ren Chen (retired), and Lefcourt invented and patented the method, which also detects fragments in fish products.

The novel technique uses fluorescence spectroscopic imaging to detect bone fragments on or near the surface of mechanically deboned meat during processing. It works by illuminating the surface of the processed meat with ultraviolet or visible light, which elicits detectable fluorescence responses from any animal bone or shell fragments present.

An objective in developing the invention was to provide a high-speed method capable of detecting the bone fragments without interfering with existing processing-line speeds or procedures. The patent could ultimately improve school lunches once the technology is licensed to, for example, meat processors for incorporation into their production lines.

"This will help ensure bone-fragment-free products," says Kim.

This research supports the USDA priorities of ensuring food safety and of improving children's nutrition and health.—By **Rosalie Marion Bliss,** ARS.

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More than 31 million children participate in the National School Lunch Program during the school year.

Celebrating 15 Years of a Healthy School Lunch Option

How about cheesy pepperoni, Hawaiian pineapple and ham, or vegetarian pizza, all piping hot and ready to enjoy? These pizzas are made with reduced-fat mozzarella cheese and are offered at the Crossroads Café—the food-service installation at Camas High School in Camas, Washington.

One technology for making tasty-but-healthy cheese was invented by Agricultural Research Service (ARS) scientists at the Dairy and Functional Foods Research Unit in Wyndmoor, Pennsylvania. It became available to schools in 1995 and is used to provide a low-fat alternative to high-fat cheese when making pizzas. The ARS team included chemists Michael Tunick, Edyth Malin, and James Shieh, physical science technician Brien Sullivan, and Peter Cooke (no longer with ARS). Other team members, Virginia Holsinger and Phil Smith, are deceased.

In 2009, more than 31 million children participated in the USDA National School Lunch Program in more than 101,000 schools and residential childcare institutions. With National School Lunch Week being observed each October, now is a good time to celebrate all healthy cheese options—produced using a variety of manufacturing methods—that are available in school lunches.

Starting in 1992, the team began exploring new ways to cut mozzarella's fat content without sacrificing its flavor or stretchy texture, especially as a pizza topping. They worked on modifying the network of the milk protein casein. The result was a mozzarella with improved storage life and only 10 percent fat—about half the fat content of regular mozzarella.

Just as important, pizza-eating students give the cheese a thumbs-up, according to school food-service director Sarah Winans with the Crossroads Café.

Martha Henry, director of food service for all schools in Tennessee's Maryville City School District, agrees. "We find that pizza is one of school kids' favorite lunches," she says. "Reduced-fat mozzarella cheese allows the students to enjoy pizza while reducing their dietary fat intake."

The USDA Farm Service Agency's Kansas City Commodity Office in Missouri began buying lower fat mozzarella cheeses in the early 1990s. Since 2000, that office has been buying lower fat mozzarellas exclusively. More than 500 million pounds of lower fat mozzarella cheeses—worth more than $800 million—have been purchased for school-related programs, according to program analysts David Leggett and Michael Buckley with the USDA Food and Nutrition Service in Alexandria, Virginia.

This research supports the USDA priority of improving children's nutrition and health.—By **Rosalie Marion Bliss,** ARS.

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U.S. Department of Agriculture
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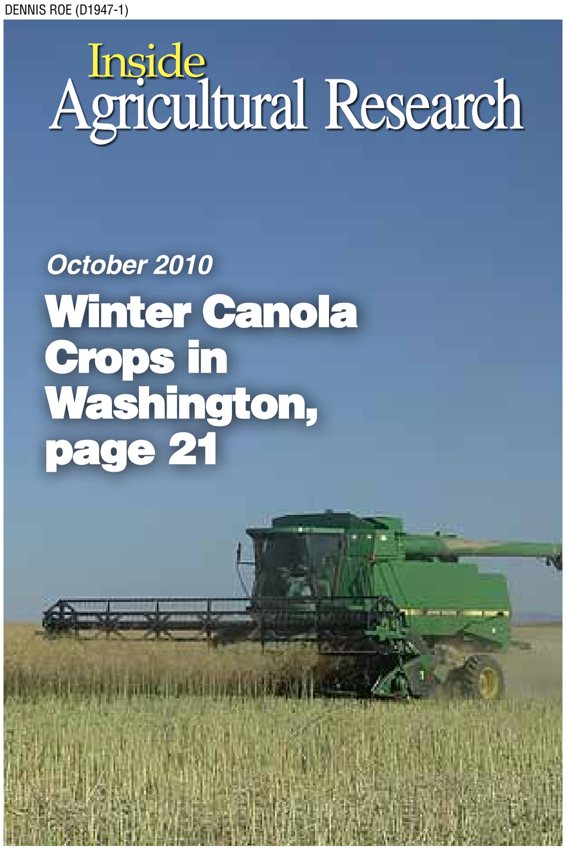

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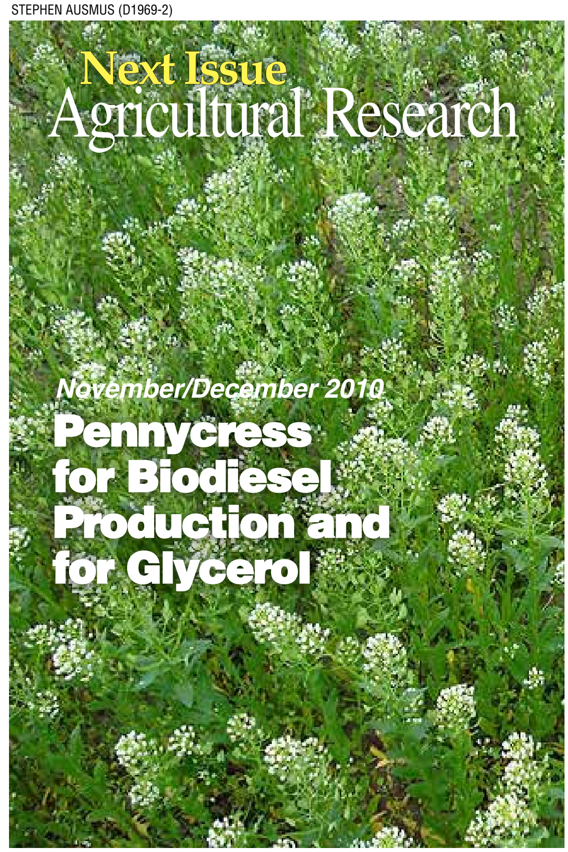

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