Superfish: The Coming Blue Revolution

by

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ABSTRACT

The Food and Drug Administration is currently reviewing applications for the commercial introduction of several species of genetically modified fish. At present, no transgenic animals have been approved for human consumption, but experts feel it is only a matter of time before approval is granted. The step could boost dwindling fish catches and provide a vital source of protein to millions. But there is growing concern among many scientists that such modified fish could lead to undesirable consequences such as human health problems and ecological disaster.

The FDA has already conducted detailed safety evaluations of the human growth hormone protein and approved its use in dairy products. The agency has also found that non-primate hormones in food are safe for human consumption. Such results will no doubt have a strong bearing on the approval of transgenic fish. At least 20 research groups in over a dozen countries are currently working to develop transgenic varieties of more than 20 species of fish. All eyes are on the U.S. as Cuba and China – the other leaders in the field – are not keen to be the first to introduce GM fish into the market. It is not known which company will be the first to get the FDA's green light or when, though some experts speculate it will be sometime in 2004. Once transgenic fish make their way into the highways of the open ocean, the FDA agrees, there can be no recall. Then it is just a matter of waiting to see if the benefits outweigh the risks.

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Located on the eastern most tip of the North American landmass, St. John's in Canada's Newfoundland province boasts a link with the sea that is older than any other city on the continent. Over a thousand years ago, the Vikings were drawn to the region's inexhaustible supply marine life. When Italian explorer – and fish trader – John Cabot landed there on St. John's Day in 1497 the abundance of fish amazed him and his crew. Cabot returned to England with tales of a "new-found land" where fish could be hauled up in buckets. By 1620, the region was a major supplier of dried cod to countries as far away as Spain and Italy. France and England fought mightily to control this vast fishery business.

Four centuries later, St. John's is now just another exotic travel getaway, a sleepy port town blessed with natural beauty, scenic drives, and historical monuments. But quiet rumblings are taking place there on the molecular level. Genetic tinkering in its laboratories is attracting the interest of the world's fish consumers and industry alike. These fruits of biotechnology promise catches of fish, the likes of which have never been seen before. If the efforts succeed and ecological concerns are allayed, genetically modified fish – bred to grow at a phenomenal speed and resist disease – promise to revolutionize the aquaculture industry and restore St. John's to its formal glory as the center of the global fish trade. But playing with nature has dangers as well. Some wonder if such attempts to remodel the work of evolution could have ecological consequences as disastrous and irreversible as a conflagration out of control.

Since the earliest days of animal domestication, humans have tried to improve the quality of their livestock through selective breeding. Though the results have been impressive, the process is generally slow and time-consuming, as it can usually take several generations of such selective breeding to produce an animal with the desired traits. But breeding efforts are imprecise, and success is mostly a hit or miss affair.

In 1866, Austrian botanist and monk Gregor Mendel demonstrated how physical traits were transferred from parent to offspring through genes. Geneticists later came to realize that if they could somehow collect the genes responsible for specific traits and selectively add them to individual plants and animals, they could make designer crops and livestock of their choice. But there was a problem. Science lacked the methods to extract, purify, and manipulate DNA, the molecule that genes are comprised of. Not until 1973 did Stanley Cohen of Stanford University and Herbert Boyer of the University of California, San Francisco, solve the problem by showing how DNA from one organism could be carefully snipped away at just the right point using enzymes as molecular scissors and be replaced with a gene from another organism. This method of gene splicing became the common means of transplanting genes from one species to another and led to the creation of the biotech industry. Scientists once had a dream, now they had the tools to realize it.

Like many other developments in science, research in genetically modified fish sprouted from an urge to find a simple answer to a simple observation. In the 1950s, Canadian scientist P. F. Scholander was puzzled to see how Arctic fish could survive in extremely cold water. He knew fish blood freezes at – 0.7 degree Celsius, but the fish he was observing were swimming in temperatures of –1.7 to –1.8 degree. How were the fish able to circulate some of the world's coldest water through their gills and not freeze to death themselves, he wondered.

To find out, Scholander and his colleagues traveled to Labrador and carried out experiments showing that some marine fish species in the cold waters of the high north developed a compound in their blood that actually lowered the freezing point of the whole fish, thereby keeping them from freezing all year round. He called this new substance "antifreeze".

The true nature of the compound, however, remained unknown for more than a decade until 1960 when University of Illinois animal biologist Arthur DeVries showed that freezing resistance in the fish was due to specific proteins that are produced in the liver and secreted in the blood to lower its freezing temperature below that of the subzero sea. "This is a very crucial adaptation," says DeVries. "Without it, the fish wouldn't survive and that in turn would have serious consequences for seals that feed on these fish, and the marine ecology in general."

But it is a bit misleading to call them antifreeze proteins, explains Duquesne University biochemist Jeffry Madura. "While the antifreeze fluid in the car radiator lowers the temperature at which ice forms, the antifreeze protein in the fish prevents ice growth till a certain temperature, thereby preventing growth of ice crystals in the gills, lesions, and blood."

Discovery of these proteins has since opened up a new field of research in preventing or reducing the damage caused by freezing. "Scientists have already used the proteins to produce laboratory strains of tobacco, tomatoes and, potatoes with improved resistance to frost damage," says Madura. And, he adds as an afterthought, "genetically engineered salmon bred to resist 'superchill' could be a boon to commercial fish hatcheries in the North Atlantic." This idea – recognized by others years earlier – is the effort to develop genetically modified (or "transgenic") fish that can survive in icy cold water and grow much faster than their counterparts in the wild. And it promises to revolutionize the way fish is harvested.

Since the early 1970s, scientists from several countries have been trying to engineer these traits in fish. Many different species have since been modified. In Cuba, for instance, Mario Pablo Estrada Garcia of the Center for Genetic Engineering and Biotechnology has modified tilapia, a common freshwater fish, to grow twice as fast. And Zhu Zuoyan of China's Institute of Hydrobiology has pioneered the development of genetically modified carp that reaches full market size almost a year earlier that ordinary carp. Eric Hallerman, a fisheries geneticist at the Virginia Polytechnic Institute and State University, notes that at least 20 research groups in over a dozen countries are currently working to develop transgenic varieties of more than 20 species of fish. The most widely publicized of these efforts is led by Aquabounty Farms of Waltham, Massachusetts. In its hatcheries based in Newfoundland, Aquabounty has so far raised more than 100,000 genetically engineered salmon that incorporate novel proteins. And plans to commercialize modified species of Atlantic salmon, Arctic charr, trout, tilapia, and halibut are currently underway. It's an impressive effort, considering that unlike the other teams, Aquabounty's foray into the field is a case of pure serendipity.

In 1974, molecular biologist Choy Hew had been appointed an assistant professor in the biochemistry department, at the Memorial University of Newfoundland in St. John's. Because of his research interest in the biosynthesis and processing of insulin in the Atlantic cod, his research lab was located off campus at the Ocean Sciences Centre in Logy Bay.

There, Garth Fletcher, a professor of fish physiology, was also researching the mechanisms of freeze protection in winter flounder. Too late in the season to get his cod from the Newfoundland inshore fishery, Choy got some shipped from Nova Scotia. The fish duly arrived and were placed in a large tank next to Fletcher's flounder. Though they were both working on different projects and different fish, sharing of the tank got them acquainted with each other, particularly when a crisis arose.

One night in February 1975, the power went out and the equipment keeping the water in the tanks at a comfortable temperature was disabled. “All my 200 plus cod had frozen to death due to the unexpectedly low seawater temperature,” Choy recalls. Biopsies of the fish showed ice in the heart. “While I was devastated, I was at the same time, the most popular guy in the Centre that day because everyone wanted cod for dinner!”

To Choy’s surprise, however, Fletcher’s flounder were still “happily swimming” in the same tank. “How had the flatfish managed to survive,” he asked. Fletcher suggested that the flounder might contain some “antifreeze molecules.” This episode marked the start of a fruitful scientific partnership aimed at understanding how the antifreeze proteins were made and how they protected the fish in harsh cold environments.

The duo’s venture into transgenic animals came six years later during a coffee break conversation with Dr. Arnold Sutterlin, a salmon aquaculturist at the Centre. Scientists have long known that growth hormones stimulate tissue growth by accelerating cell division so that new cells can be made. The stimulation increases appetite, competitive ability, and ultimately growth rate. But there is a problem in winter. Limited food supply means there isn’t enough to satiate the fish’s hunger, leaving it exposed to frigid temperatures and numerous predators. “In the past several years, presumably as a result of global warming, temperature in the New Brunswick region declined sharply. The salmon industry was hit hard because the cages were covered with ice,” says Choy.

To survive such dangers, evolution has armed several species of cold-water fish with a clever adjustment. When the temperature falls below a certain mark, the nervous system sends a signal to stop production of the growth hormone. No more a slave to hunger, the fish go on a diet, stop growing, and swim to deeper water where they wait out the winter. Sutterlin wondered over coffee whether it was feasible to help the Newfoundland salmon industry by producing a freeze-resistant salmon that would maintain continued growth through the winter.

The solution seemed straight forward: provide the salmon with a set of antifreeze genes to help it survive much lower temperatures and hence keep the growth hormone flowing. But it was not that simple. Growth hormone genes are normally expressed in the pituitary gland and regulated by the brain. The original gene is programmed to turn off in extremely cold weather and turn back on in summer. But when fish are modified to withstand freezing by simply adding an antifreeze gene, the growth hormone gene does not alter its response. The only way past this seasonal arrangement is to sidestep the sensory thermostat and have a growth hormone produced at a different location. To do this, scientists deliberately picked an antifreeze protein gene, which induced production of growth hormone in the liver.

In such fish, when the native growth hormone is closing down at the onset of winter, the antifreeze protein acts as a genetic switch, or promoter, to turn on the added growth hormone gene in the liver. So in summer, the growth hormone is under the control of its inherited gene, while in winter it comes under the control of the inserted gene. Here was a winning formula. Instead of just one season, the fish keeps growing all year.

“It was a challenge that a molecular biologist like myself would gladly accept. I was both naïve and excited and took the bait,” says Choy. The scientists began by matching a promoter from the ocean pout with a growth hormone gene from the Chinook salmon. The ocean pout promoter was chosen because it is expressed predominantly in the liver throughout the year. Initial results were spectacular: compared to salmon in the wild, the genetically modified salmon matured to market size in half the time.

Says Fletcher: "The genetic material inserted into a modified fish typically contains both a growth hormone gene and a DNA sequence, or promoter, that controls the gene." These sequences tell the gene when to turn on and off. "Selecting the right promoter DNA sequence allows scientists to "trick" the fish's cells into producing growth hormone throughout the year, instead of just the summer months. The secret to achieving faster growth in the genetically modified salmon lies not in the growth hormone but in the promoter that controls it," adds Fletcher.

The modified fish are termed transgenic because they contain copies of a gene sequence that have been artificially added. This novel DNA is referred to as the transgene. Fletcher explains: "A transgene is basically a segment of DNA containing the desired gene and the promoters. At the end of the gene a bit of DNA - called Poly A - is added to mark the terminating point." The whole transgene can be thought of as a small computer program in which the promoter represents the initial conditions that control how the main body of instructions, in this case the gene, is executed. The Poly A tail acts as an "end" statement.

To make a transgenic fish, though, enough copies of this small program, or "gene construct" as it is technically called, must first be produced. This is accomplished by inserting the construct in a bacterial plasmid. These are circular strands of DNA that are not part of the bacterial chromosome, and multiply independently within the bacterial cell. The bacteria are then cultured to make billions of copies.

At this stage, the plasmids are separated from the bacteria and the circular strands are sliced to obtain linear gene cassettes that are ready to be injected into newly fertilized fish eggs. But scientists have to make a compromise on how many cassettes to inject into each individual egg. The number must be high enough to ensure a reasonable percentage of transgenic fish and low enough to ensure survival of the embryo. Studies show that the optimal number to be injected is about a million.

The chance of success for just one among this army of a million to land on an appropriate segment of the fish genome and express itself in the desired way is about one or two in 10,000 embryos. "It doesn't always work the way we want it to," says Fletcher. "So out of those thousands and thousands of eggs you transform to start with, only a relatively small number – about a few hundred fish – had successfully integrated the transgene." Once incubated, the successfully engineered fry are allowed to grow and reproduce. Mendelian inheritance kicks in after four generations: that's the point at which the added gene is reliably passed on. At that stage, fish with the desired traits are selected and bred further.

The decision to commercialize this procedure came about by another chance event. Up until the early 1980s, most of this research was still being conducted in the labs at the Ocean Sciences Center. In 1987, Elliot Entis, a wholesaler who sold fish from New England to the Caribbean, chanced upon the scientists' work through newspaper reports. Intrigued, he wanted to know if the research could help in better preserving fish and in increasing the shelf life of frozen fish stocks. Talking to Fletcher, he found there were indeed various uses for the antifreeze proteins, not just for fish but in medical applications as well. For instance, the proteins could be used in freezing the tissue around the heart so that it could be protected during open-heart surgery. One could also target a tumor by freezing it and yet protect the surrounding regions with the antifreeze proteins.

A decision was made to form a company called AF Protein. "After the deal had been made and everybody was shaking hands, Fletcher casually mentioned, 'by the way I've got a tank of fish you might want to see, they grow like hell'. Elliot took one look at the fish and decided to form another company. And that was how Aquabounty Farms, a subsidiary to AF Protein was set up," says Joseph McGonigle, the company's vice president.

However, before these fish become the first genetically modified animals to reach dinner plates, Aquabounty has to get a clearance from the Food and Drug Administration (FDA) that the fish will not endanger human health or other fish. That may not be a tough task for the company as most proteins are digested after consumption and there is no evidence yet that transgenic fish cause allergic reactions. Moreover, the marketing of transgenic fish requires only the FDA's approval; genetically modified plants, on the other hand, have to be approved by the FDA, the U.S. Department of Agriculture, and the Environmental Protection Agency.

McGonigle says Aquabounty has supplied all relevant information the FDA might need in addressing potential health risks. "This is not a yes or no document. The FDA keeps questioning us on the various concerns it has and we reply with experimental data to allay those fears. Once we have satisfactorily cleared all doubts, the FDA will send us a letter saying the agency has no more questions," says McGonigle. If and when it gets that green light, Aquabounty hopes to focus undivided attention in marketing the fish to the world's largest consumer of farmed fish: the United States. One of Aquabounty's flagship products is the transgenic Atlantic salmon. Known by the trade name 'Aquadvantage Salmon', these fish are capable of growing four to six times faster than standard salmon raised under the same conditions. These new salmon reach desired market size and weight in 18 months instead of 36 months.

"Just look at the world, from India to China," says McGonigle, making a case for Aquabounty's vision the Blue Revolution. "There is a massive development in fish farming around the world. Countries such as Bangladesh, Thailand, Vietnam, and China are making giant strides in fish and shrimp aquaculture. China has pretty much cornered the tilapia market while Thailand is turning into a powerhouse of shrimp farming. And Vietnam is diversifying into many areas of aquaculture. This is our target market segment. Ours is not a fish company, it is a biotech company and we plan to sell eggs of our transgenic fish to aquaculture farms," says McGonigle. Aquabounty hopes to become the Monsanto of the sea.

Global aquaculture statistics seem to support the business plan. In a report on "The State of World Fisheries and Aquaculture," the Food and Agriculture Organization (FAO) noted that world capture fisheries production decreased from 95.4 million tons in 2000 to 92.4 million tons in 2001, while aquaculture production increased from 35.5 million tons ($ 52.1 billion by value) in 2000 to 37.9 million tons by weight ($ 55.7 billion) in 2001. That's an increase of $ 3.6 billion in the space of just a year. Indeed, aquaculture production is expected to nearly double over the next two decades and by 2020, developing countries are expected to contribute nearly 80 per cent to this increase.

Though transgenic technology could lead to desirable traits in fish and hence boost the aquaculture industry, supporters stress the importance of using genetic engineering to address the global hunger problem. Essential fatty acids, primarily sourced from aquatic organisms, have been a major driving force behind our past evolution. The FAO estimates that fish are an important source of protein, especially in developing countries, and account for 20 percent of animal-derived protein in low-income, food-deficit countries. Scientists speculate there is a direct link between these life-sustaining molecules and our future. But the availability of these fatty acids is in jeopardy. The FAO says more than 70 percent of the world's fisheries have either been depleted or are nearing depletion. The problem is compounded by the growing world population, estimated to jump from 6 billion to almost 9 billion by 2050.

Says Fletcher: "It is critical that we develop alternative methods to ensure future quantities of fish. Transgenic fish aquaculture appears to be the only viable means of meeting future demand."

Alleviation of world hunger appears to be the strongest argument for the introduction of genetically modified foods. And its supporters have history on their side. In the 1940s, efforts to develop a high-yielding variety of wheat that was resistant to many plant pests and diseases led to dramatic increases in crop yields across the developing world. The use of genetic engineering to produce high-yielding disease resistant strains of fish is viewed as a natural continuation of the Green Revolution. The promise of a corresponding Blue Revolution is implicit. But hunger is not the only driving force. Transgenic fish, say supporters, have a tremendous potential to also advance human health care.

For instance, researchers at a Florida-based company, Aquagene, are trying to develop a variety of transgenic tilapia to make human coagulation factor VII. This is a blood coagulating protein used to treat such disorders as end-stage liver disease and hemophilia, the oldest known hereditary bleeding disorder. It is estimated that there are about 20,000 hemophilia patients in the United States and that each year about 400 babies are born with this disorder. The severity of the disease is directly related to the amount of protein essential for clotting the blood. About 70 per cent of patients have less than one percent of the normal amount and thus have severe hemophilia. With the help of researchers from nearby University of Florida, AquaGene splices the human gene for factor VII into fish genes. Producing purified factor VII protein more cheaply for future clinical use is still a few years away, but success would help thousands currently unable to afford the high cost.

The use of transgenic fish as indicators of polluted water is another application being explored by environmental scientists. Nearly 100 years ago, miners carried caged canaries down to their mines to alert them to the presence of deadly carbon monoxide gas. In a way, some transgenic fish can be thought of as canaries of the water, alerting humans to the presence of polluting heavy metals and harmful chemicals.

There are a number of chemicals in the water that kill fish and make humans sick. Some of these chemicals act in subtle ways, not making us immediately sick but instead inducing genetic mutations that can cause cancer and other deadly diseases in the long term.

Experts in Singapore have spliced genes from jellyfish and sea anemones into zebra fish to make them turn fluorescent red or green when they come into contact with pollutants. The idea is to produce commercially viable zebra fish that can be used as a simple, cheaper alternative to current systems used to test for pollution. These so-called “glofish” are currently being marketed in the United States by Texas-based Yorktown Technologies and have already become the first transgenic pet to be sold in the country.

Says Singapore's National University's Zhiyuan Gong, who originally created the fluorescent fish: "The most important benefit is that these fish can help develop an online system to monitor water quality. Compared to conventional methods of installing chemical sensors and measuring the pollution, the live fish can tell us the biological effect of the pollutants."

At present, Gong says his fish can only indicate the presence of estrogen and heavy metals. Although red and green are the only colors that have been produced in the zebra fish thus far, Gong and his team of researchers believe the fish can be engineered to produce as many as five colors, each color indicating a different pollutant. Critics point out that some fish are known to change color to attract mates and that such modified fish could interrupt natural behavior in the species. But Gong does not seem too worried about such a disruption. The research is being extended to goldfish and koi, creating a stir in the billion dollar annual business in home aquariums. Scientists have already equipped goldfish with cold tolerance and there is widespread concern that this fish, which has already established itself in some inland water bodies in the U.S., could soon turn into a nuisance species.

Such possibilities have served to heighten a sense of mistrust about transgenic fish in certain sectors. At the heart of opposition to genetically modified organisms lies the belief that transgenes could confer undesirable traits and properties in the host animal or plant. But Professor Norman Maclean at the School of Biological Sciences at the University of Southampton dismisses such concerns and claims that all the genes used to date in creating transgenic fish are safe to eat. The same goes for the hormones, he says, since they are digested in the gut. To ensure the fish are safe, he suggests experts should more carefully scrutinize all components of the gene construct, including the promoters and the coding sequence.

Clearly, caution is the key. The Grocery Manufacturer's Association notes that nearly 70 per cent of all foods on the shelves of grocery stores in the United States today contain some component that was derived from a genetically modified organism. The first genetically modified product – the enzyme chymosin – was approved for use in 1988. Nearly 90 per cent of all cheese made in the U.S. is derived from genetically modified chymosin, which is traditionally obtained from the extracts of calves stomachs. The list of FDA-approved genetically modified foods include such common foods as soybeans, corn, rice, potatoes, tomatoes, squash, papaya, canola, cantaloupes, and sugar beets. And the list extends to processed food as well. Indeed, each day Americans consume large quantities of genetically modified food, from the corn syrup in Coca Cola and the potatoes in McDonald's French fries to the soy in Nestle chocolates.

Food safety experts are still wary and worry that novel proteins in transgenic fish could induce adverse immune reactions or allergies in humans. Though a protein expressed by a transgene is no different to the immune system from any other protein, there is reason to exercise caution, they say. Studies on allergic reactions to proteins in the food are limited, but scientists do know that different individuals may be allergic to different parts of a protein. "The National Academy of Sciences in a recent study acknowledged that novel proteins could trigger allergic reactions," says Tracie Letterman, fish program director at the Center for Food Safety (CFS). But of greater concern to the Washington D.C-based environmental advocacy group is the larger issue of regulatory oversight. "We are concerned at big gaps in the regulatory process and would like Congress to step in and address the issues." For instance, points out Letterman, the FDA treats transgenic fish not as a food but as a new animal drug. This, she says, translates into an opaque and secretive process. "We will know what fish are approved and what tests were conducted to assess their safety only after the fish have been approved," notes Letterman.

Her views are echoed by Rebecca Goldburg, senior scientist with Environmental Defense. "The FDA may not regulate transgenic fish except under food safety provisions of the Federal Food, Drug, and Cosmetic Act [FFDCA] but the agency's policy for applying the Act is unclear and in dispute internally. Also, regulation will take place out of the public eye, since the FFDCA requires that the agency not release information about pending drug approvals."

John C. Matheson, senior regulatory review scientist with the FDA's Center for Veterinary Medicine, defends the agency's decision. "The FDA had decided to view transgenic fish as a drug after detailed discussions within the agency." He points out that the genetic modification in the fish is not used to change the food quality of the fish but rather to alter the growth of the fish, a modification that falls under the definition of a drug. "As for the secrecy charge," he explains, "we are trying to be more open, as much as rules can allow us to be. But we have to protect trade secret laws as well."

A more serious question to be addressed, according to the CFS, is the competency of the FDA in dealing with the potential ecological risks associated with accidental escape of transgenic fish into the wild. Letterman feels that the FDA does not have the expertise to deal with environmental problems and that the task is best left to government agencies such as the Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS).

Others agree. "Let's face it," says Virginia Polytechnic's Hallerman. "The FDA is not the best agency to deal with environmental and ecological problems. Just look at the glofish issue. There seems to be no clear jurisdiction on who will regulate the fish." The FDA, however, finds no cause for alarm. A statement by the agency in late 2003 said: "Because tropical aquarium fish are not used for food purposes, they pose no threat to the food supply. There is no evidence that these genetically engineered zebra fish pose any more threat to the environment than their unmodified counterparts, which have long been widely sold in the United States. In the absence of a clear risk to the public health, the FDA finds no reason to regulate these particular fish." Last January, CFS filed a lawsuit against the FDA to block the sale of the glofish and sought a court order stating that transgenic fish are subject to federal regulation and cannot be sold without proper authorization.

Hallerman calls for responsibilities in assessing the risks from transgenic fish to be divided between the Fish and Wildlife Service (for fresh water fish) and the National Marine Fisheries Service (for marine fishes). One of the problems, he notes, is that the states have no say in the regulation process, even though they have greater local expertise. "Surely Florida Fish and Game would have more expertise and knowledge of its waters than the FDA."

The FDA is, in fact, cooperating with the FWS and the NMFS, answers Matheson. "Both agencies are free to make suggestions," he notes. "But they have no say on any issue."

Among the potential risks associated with transgenic fish, the one that is of greatest concern is horizontal gene flow. Unlike vertical gene flow, which is the natural transmission of genes from parent to offspring, horizontal gene flow is associated with the ability of genes to jump between species. This kind of gene transfer is difficult to observe in laboratory tests, and tests held in controlled settings can often provide skewed results. To get a more realistic understanding of the possible impacts of transgenic fish on the environment, scientists decided to conduct field trials. However, this was not a simple and straightforward step. Research teams first had to convince federal authorities that all precautions to prevent escapes were taken and that there was no threat to the environment.

Early in 1989, the biotechnology review board of the U.S. Department of Agriculture approved the first field tests involving a genetically engineered fish. Researchers at Auburn University in Alabama were permitted to introduce fish enhanced with growth hormones into a test pond at the university. Rex Dunham, an associate professor of fisheries and allied aquacultures at Auburn, commenced trials two years later with channel catfish modified with growth hormone genes from the rainbow trout attached to a promoter from the Rous sarcoma virus. These fish were the result of collaboration between researchers at Auburn and at Johns Hopkins University in Maryland. Since fish containing growth hormone genes had been shown to grow significantly larger under laboratory conditions, the findings held potential commercial significance for the fish farming industry.

Precautions were stringent. The fish were kept in a contained earthen pond, with a series of barriers to prevent escape into open waters. As a foolproof measure, a special mechanism was developed to introduce poison into the water, in case a fish managed to get past even one of the barriers.

For comparison, the scientists added unmodified catfish into the population of transgenic fish. Initial results suggested a 41 per cent higher growth rate in the transgenic fish over the unmodified test subjects. Says Dunham: "You can add a hundred or thousand growth hormone genes and it will make no difference. The key to this impressive growth was due to the viral promoter." But use of such viral promoters – the Rous sarcoma virus is similar to HIV that causes AIDS – is not viewed favorably and Dunham declares he has no plans to commercialize his catfish.

The Auburn tests highlighted another interesting behavior. While both groups of fish have similar abilities in avoiding predators, more transgenic males and non-transgenic females were able to mate successfully in a competitive mating situation. There was only one conclusion: if the transgenic fish were to somehow escape, the transgene was likely to enter and linger in wild channel catfish.

Field trials of transgenic fish were also conducted by Jorgen Johnsson, an animal ecologist at the University of Göteborg, Sweden. In the experiments, repeated over two years between 1999 and 2001, Johnsson's team studied the growth rates and behavioral responses of brown trout in experimental streams. But these were not transgenic trout, because the Swedes were not permitted to release and study genetically altered fish in outdoor streams. So the team did the next best thing: it equipped the fish with implants that slowly released growth hormone. Over a year the 'transgenic' fish grew 20 per cent faster, ate more, and showed less fear of predators.

"We don't know if our study accurately mimics transgenic fish or how successfully transgenic fish can compete with wild fish," says Johnsson. There could, however, be some disruption to local trout populations, he admits, and stresses the need for a thorough risk assessment over the next five to ten years. "With our present state of knowledge, I am very concerned about accidental release of transgenic into the environment," he says.

All these preliminary findings, however, were soon overshadowed by the next study, which stunned the scientific community. In the same year as Johnsson's tests, two scientists at Purdue University decided to study the ecological consequences of releasing transgenic fish into wild fish populations. Though previous studies suggested that such a release could push local species to extinction, many scientists at the time believed this outcome was unlikely. Transgenic fish were thought of as evolutionary novelties that lacked the ability to survive without artificial support. The Purdue study forcefully reversed that assumption.

For the experiments, animal scientist William Muir and biologist Richard Howard chose the Japanese medaka, a fish known for its short life cycle. The medakas were modified by inserting a gene construct consisting of the human growth hormone driven by a growth promoter from the salmon. A series of aquarium tests showed that the larger transgenic medakas enjoyed a four-fold advantage in mating over smaller males. Muir and Howard plugged the results into a set of mathematical equations to predict the effect of introducing such transgenic fish into a wild population. To account for varying results from different transgene lines as well as to make the study applicable to other organisms, the mathematical model comprised a number of fitness parameters, such as sexual maturity, survival rate, mating success, number of eggs, and age at sexual maturity. The equations spit out three possible outcomes.

In the first scenario, called the purge scenario, when the net fitness of the transgenic fish is lower than that of the wild fish, natural selection quickly "purges" any transgenes inherited by the wild fish. Under this model, genes of the transgenic fish would ultimately disappear from the gene pool, though there would be some effect on the wild fish, depending on the population size. A small colony would be seriously threatened.

The second result was cause for some concern. When the net fitness of the transgenic fish was equal to or higher than that of the wild fish, a gene flow was likely to occur. The transgenes then spread throughout the wild population. Studies showed that in this "spread" model, net fitness was greatly influenced by age at sexual maturity. This was found to be consistent with studies that showed Coho salmon with growth hormones reaching sexual maturity earlier than the wild fish.

But it was the last scenario that created headlines around the world. If the previous results were like buzzer alerts, the third outcome was like a fire siren. It suggested that mating advantage enjoyed by the transgenic fish would help the transgenes spread rapidly into the wild population. However, each subsequent generation carrying the new genes would have a lower survival rate. That is because under controlled conditions the transgenic medaka were 30 per cent more likely to die before reaching sexual maturity and that would ultimately decimate the entire population.

By plugging the net fitness parameters and other experimental data into a computer model, Muir and Howard found that if just 60 transgenic medaka were introduced into a population of 60,000 wild medaka, it would take as little as 40 generations for the entire population to be wiped out. "This resembles the Trojan horse," says Muir. "It gets into the population looking like something good, and it ends up destroying the population." Fittingly, Muir and Howard called this startling conclusion the Trojan gene effect.

"The Trojan gene was an accidental finding," adds Muir matter-of-factly. "One does research where there are funds. Our main incentive was a biotechnology risk assessment grant from the Department of Agriculture to study the effects of all possible transgenes now and in the future. Without the incentive there would have been no study." He also cautions against accepting the test as the final word. The question that needs to be answered, he says, is will the frequency of escape of fertile fish fall under acceptable risk levels. Those levels are determined by both the potential harm and the potential for spread. "Neither of these has been demonstrated with this genetically modified fish. We have a lot of theory, but no real data."

The study remains a topic of great debate. From the cold reaches of Newfoundland to the hot humid interiors of China's Hubei province, Muir's name is instantly recognizable. And his findings have divided fish geneticists into supporting and opposing factions. CFS' Letterman claims Muir's findings are the "clearest representation of the dangers of rushing to approve transgenic fish, without adequate safety tests," but other experts are not so sure. Robert Devlin, a biologist with Fisheries and Oceans Canada, the premier government agency responsible for the conservation and sustainable use of fisheries resources, notes that "computer models are a very useful tool in exploring outcomes from several different variables, but they are not a final answer to risk assessment of transgenic fish." Others argue that the study is too simplistic and does not tell the entire story. "The experiments were conducted in an aquarium setting and had no predators. That makes it highly artificial," says Auburn's Dunham. Critics also suggest that results from experiments on a small fish such as the medaka cannot be plugged into a model for larger fish such as the salmon, which thrive in different conditions. "It would be erroneous," says FDA's Matheson. "We do not know if transgenic male salmon have a mating advantage over non-transgenic males. The advantage that Muir talks about may only be relevant to the medaka fish," says Fletcher.

Muir has heard similar arguments many times before and gives a practiced reply: "One is not extrapolating a model fish. The theory is species independent, just as the mathematical principles of physics and gravity apply to all matter, although it was perhaps discovered with an apple. There are always exceptions to any rule, the exceptions do not invalidate the rule, they just cause exemptions, such as special relativity. Ours is the only model based on Darwin's theory of natural selection."

Keeping in mind the dangers of escape, a report released earlier in January this year by the National Research Council of the National Academy of Sciences (NAS) asked transgenic fish researchers to consider how induced sterility – or bioconfinement, as it is known – could prevent transgenic animals from escaping and breeding with wild populations. The solution involves setting up aquaculture farms in land-based locations, far away from major waterways, thereby reducing the chances of an escape. However, this is an expensive proposition and puts land-based farms at a competitive disadvantage vis-à-vis aquaculture farms in other countries, where the fish pens are anchored out at sea. These floating sea pens require little maintenance and can house thousands of fish at a time.

Aquabounty has its own defense against the Muir study and the NAS report. It claims that its fish will be made biologically sterile before being released into the market. Sterility is achieved through a process called chromosome set manipulation to create sterile fish known as triploids. These are individuals bearing three sets of chromosomes (two from the mother and one from the father) instead of the usual two sets (one from each parent), and are generated by interfering – with the help of electric shocks or temperature variations – with normal cell division just after the eggs are fertilized. Triploids do not develop normal sexual characteristics, and since their eggs or sperm have an extra chromosome that is unable to match itself to its opposite, reproduction is stymied.

"At present we have male and female transgenic salmon – this allows us to generate offspring," says Fletcher. We are working on producing an "all female" stock which will comprise regular females which produce eggs, and sex-reversed females which will produce sperm. When you have such a stock you can fertilize the eggs with sperm from a sex reversed female and all of the offspring will be genetically female. To produce these sterile "all female" offspring for commercial culture, we pressure treat the fertilized eggs and render them triploid."

The aim of this bizarre sexual makeover is simple: produce only sterile females. The company plans to submit details of the sterilization verification process to the FDA to assure the agency of complete sterility in the fish. McGonigle claims that even if 100,000 female transgenic salmon were to escape, only 12 of them would be likely to return to their spawning grounds and open the possibility of genetic pollution. But even among these 12, only two salmon would be likely to succeed in reproducing. And two fishes, says McGonigle, can do no harm. "With an assured sterilization process in place, no fertile fish will ever be sold for use in commercial aquaculture."

But complete sterility in a group is a tall claim, say some scientists. "I just wish it were so easy," notes Hallerman. "It is very easy to test 10 fish and claim hundred per cent success. But each fish has thousands of eggs and you have to test each of the eggs over several years. I think they have either resisted conducting the hard tests or are simply not reporting the results."

While researchers agree with Muir's cautionary advice to not jump to conclusions, evidence from the field shows how easily the Trojan gene effect can become a reality. Hallerman points out that even in the most secure aquaculture farms, approximately two per cent of the fish manage to escape. "Most farms have thousands of fish, while the area near it probably has only few hundred fish. It is not difficult to see how the wild population can be easily overwhelmed."

For instance, federal wildlife officials and environmentalists are trying to save Maine's $ 65 million salmon aquaculture industry from complete ruin. That is because in 2001, nearly 100,000 farm salmon escaped after a fierce storm damaged their holding pens. Many escapees mated with their wild cousins. Subsequent generations were unable to find the way back to their spawning grounds. "If these were sterilized transgenic fish and the sterilization process was only 99.9 per cent effective, then 100 fertile transgenic fish would have escaped. Can you think of any biological procedure that is just 99.9 per cent effective," asks Purdue's Howard.

Wildlife experts feel such escapes could prove disastrous to the dwindling populations of wild salmon. Once abundant in the Hudson River in New York and parts of Canada, the Atlantic salmon are now largely found in Maine. And there too, the numbers have drastically declined. Historic records shown that in the 1800s, almost half a million adults returned to all U.S rivers. The National Research Council estimates that in 2002, only 871 fishes returned.

The NAS also has voiced concerns about the sterilizing process. The technique, the Academy said in its report, "cannot guarantee 100 per cent sterility." That means some escapees will still be capable of breeding and passing on their tampered genetic legacy into the wild.

But even if complete sterility is achieved, there are additional problems. Scientists fear that transgenic fish will outlive their wild counterparts and continue to outcompete them for the limited food supply. Mating advantage is a more serious concern. Wildlife biologists have for a long time known that sexual preference dominates Charles Darwin's classic theory of survival of the fittest. Muir likes to illustrate his point with an example of the male bird of paradise with its long cascades of gloriously colored plumage. "The male bird of paradise with the longest, thickest tail attracts the most females. Subsequent offspring also exhibit the long tail and also compete well for females. Unfortunately, the birds with the biggest tails also have the biggest problem escaping from predators who appreciate large birds pinned in place by their plumage. Obviously the bird with the most sex appeal is the also the worst choice as a fit mate. Not unlike high school, some might say."

Studies show that the testosterone levels of triploid fish are comparable to that in wild fish. Therefore, the sterility notwithstanding, these fish will still indulge in mating behavior, and research shows that fish in the wild tend to prefer larger mates. Since transgenic fish are bigger, the larger sterile fish will have a mating advantage over other fertile males. Scientists say this mating scenario, in which fertile females chose the larger – but sterile – males over smaller fertile males, could seriously threaten wild populations.

Late last year, the Purdue scientists made another intriguing discovery while watching an intense mating game unfolding in their aquarium. The transgenic males, by virtue of their larger size, were elbowing out the smaller non-transgenic males found in the wild. "They were getting a lion's share of the matings: more than 75 per cent," says Howard. But the underdogs were not about to give up that easily. Like guerrillas, the wild males resorted to hit-and-run mating tactics to produce their own offspring. The tactics were twofold: either disrupt a mating and gain access to the female or join in with other transgenic males while they are mating and release their own sperm.

Says Howard: "After I saw this behavior by the wild-type males, I was curious about how many young the sneak males could produce, as in some species they can be more successful that the dominant males." The scientists added two more parameters to their previous mathematical model: the chance of transgenic matings that would include such sneak attacks and the degree of success such attacks had in fertilizing the eggs. The scientists wondered if nature had found a way to preserve its wild heritage. Could the sneak attacks somehow dilute the mating advantage enjoyed by the larger transgenic males? But the hope was short-lived. "Based on our data, our model predicts that the sneak strategy is not strong enough to stop the Trojan gene," says Howard.

The findings provide yet another stark reminder of the risk of commercializing transgenic salmon. "I didn't know that medaka did this; however, I knew that several other fish species, including many salmon did. Thus, this was a relevant extension of the research, particularly for salmonids," notes Howard.

The National Academy of Sciences recently highlighted yet another concern. Current research on the creation of sterile transgenic animals assumes that the animals are dioecious, that is male and female reproductive organs are in separate individuals and each individual maintains the same gender for life. However, there are exceptions. Some synthetic chemicals and natural plant compounds are known to cause reproductive and other health problems in wildlife and laboratory animals. The NAS points out that aberrations in reproduction such as hermaphroditism (individuals with both male and female organs) and parthenogenesis (reproduction by development of an unfertilized, usually female, germ cell) are not uncommon among certain fish, mollusks, and crustaceans. So bioconfinement methods based on thwarting sexual reproduction would simply fail in this case. Scientists have already found at least one species of finfish awaiting a transgenic makeover that is reported to exhibit hermaphroditism. Researchers fear that such self-fertilizing transgenic fish, which do not require the physical union of a sperm to reproduce, pose a grave danger. The escape of just one fertile individual could result in the formation of an entirely new population. But McGonigle says there are errors in the Council's report. "They clearly don't have a full grasp of our proposal and the effectiveness of our technology."

The dispute over the ecological safety of transgenic fish has already claimed its first victims: two companies that licensed A/F Protein's gene insertion technology in the 1990s. Scotland's Otter Ferry Salmon and New Zealand's King Salmon Company had to scrap their transgenic salmon research after unfavorable publicity regarding deformed fish. The media attention has drawn considerable political attention as well. In December 2002, even as the FDA was considering an approval for the transgenic fish, Washington state issued a ban on transgenic fish. A similar ban is effective in Maine as well, which means no salmon farmer is allowed to rear transgenic Atlantic salmon.

Following this lead, California last October became the second state to formally ban salmon farming in its waters and is considering outlawing genetically modified fish as well. On the case of the glofish, however, the California Fish and Game Commission recently agreed to formally consider allowing sale of the glofish in the state. Presently, eight states have some level of restriction for the introduction of transgenic fish, and reports suggest several other states are planning similar restrictions. The wide publicity has caught the eye of several major food outlets, which have decided not to sell transgenic fish, if and when they are approved. The fears are not restricted to a few American states and restaurant chains. International groups are voicing concern as well.

In June last year, the North Atlantic Salmon Conservation Organization (NASCO), a seven-member international organization formed to promote the conservation and management of salmon stocks in the Atlantic, adopted a new resolution seeking to minimize potential dangers from transgenic aquaculture. Terming the use of transgenic salmon a "high-risk activity," NASCO urged members to provide all information necessary to demonstrate that the move will not have an adverse impact on wild salmon stocks or lead to irreversible change.

With FDA approval looking increasingly likely and consensus on the issue appearing like a distant dream, some experts in academia and industry suggest the decision to eat transgenic fish is best left to consumers. This could be done through labeling, allowing a consumer at the grocery store to decide for himself whether or not to consume a genetically modified fish. Public support for labeling genetically modified food is widespread. According to a recent study by the Food Policy Institute at Rutgers University, 94 per cent of those surveyed said they would like to a label marking genetically modified food. "If the efforts to block the approval are unsuccessful, I am sure the next effort will be to require labeling. Thus far, however, I haven't heard of any real campaign to make sure these fish are labeled. In the long run, labeling might benefit companies and their products will sell more, as it implies trust and honesty," says Hallerman.

But under U.S. law, food products developed through biotechnology are not required to be labeled unless the nutritional content of the food has been changed, the product contains a known allergen, or genetic modification has changed the food so much that it is considered a new food product altogether. For its part, the FDA believes that labels can be misleading. To the agency, a label implies a certain food is either better or worse than other food products.

But labeling is already common practice across Europe and Japan. With aims to corner the global aquaculture trade, Aquabounty must take that into account. The company says it will go ahead with voluntary labeling and soon come up with an easily recognizable logo for its Aquadvantage Salmon.

Along with environmental and political concerns, there is also an ethical question on whether it is acceptable to genetically engineer animals at all. In a monthly newsletter of the Pew Initiative on Food and Biotechnology, Professor Neal First of the Endocrinology and Reproductive Physiology at the University of Wisconsin, Madison, said he believed genetic engineering was a more targeted approach to centuries of changes in animals due to environmental conditions and selective breeding. So in essence, the technique is just another extension of breeding. But the change in the animal, adds First, a former director of the USDA's National Animal Genome Mapping Program, must serve a human need, the modification not carried out for frivolous or trivial purposes. These would be defined as experiments that do not advance our health. A case in point is the creation of the glofish for use in aquariums, or the "enviropig," which is a pig genetically engineered to produce less phosphorous in its waste.

But Marlene Halverson of the Animal Welfare Institute - a non-profit organization set up to reduce pain and fear inflicted on animals by humans - offered a contrary view: that all animals have the right to live and be left alone and that the world wouldn't go hungry without transgenic animals. Though she agreed with First's view of biotechnology as an extension of slow conventional breeding techniques, she felt that was not reason enough to harness the new knowledge. The caution, she says, is borne out by some problems with the technology. For instance, pigs modified to be lean tend to have a longer body, which in turn causes the animals to go lame. The technology increased the risk of miscarriages, Halverson noted.

So do we need transgenic fish at all? In the Pew newsletter, Anne Kapuscinski, a fisheries and wildlife professor at the University of Minnesota, admits there is no sweeping argument either in favor or against transgenic fish. Different people will be affected in different ways and there will be large trade-offs involved. For instance, fast growing salmon could help commercial farms double their yields each year and soon supply could exceed demand, resulting in lower prices, which in turn could put salmon fishermen in regions like Alaska out of business and cripple the region's economy.

In reply to Aquabounty's argument that global fish catches are declining and transgenic fish could supply the requite protein source, Kapuscinski said that declining stocks only mean local populations will be more vulnerable to a hostile takeover from genetically modified fish. But others see the technology as a tool that humans can use to manipulate their resources. Offering a counterpoint, James Carlberg, president of Kent Seatech Corp, wrote in the newsletter that since fish in aquaculture farms use a large amount of feed generated from wild fish, transgenic fish – due to their ability in consuming food more efficiently - would be a better bet as they would eat less and produce even less waste.

Amid these claims and counterclaims, efforts to commercialize transgenic fish lines are quietly going ahead. Aquabounty has submitted an application to the FDA seeking approval to market eggs of transgenic Atlantic salmon to commercial aquaculture producers. Matheson refused to comment on when, and whether, Aquabounty would get an approval. On being told that Aquabounty's Garth Fletcher had claimed in a recent interview to the BBC that the company might get an approval by the end of the year and start selling its product by 2005, Matheson simply said: "They may have underestimated the time line."

Researchers in Cuba and China are reportedly close to developing transgenic varieties of tilapia and carp. At the Center for Genetic Engineering and Biotechnology in

Havana, scientists have managed to genetically modify the tilapia's stomach to make it more efficient in absorbing nutrients from food. Auburn's Dunham feels Cuba is the perfect place to introduce transgenic fish into the market. "They're an island nation. Even if the fish manage to escape, the freshwater tilapia have nowhere to escape," he says. But even after extensive tests to evaluate food safety and risks of escape, the tilapia is currently not being marketed for consumption. The general feeling among the scientific community is that Cuba wants to avoid a public relations disaster by becoming the first nation in the world to release transgenic fish into the market. Says Cuba's Estrada: "We have a regulation process like that of the FDA but it is more similar to European guidelines on GMOs. We expect to present our documentation to the regulatory institution in two years. I hope that the transgenic salmon gets approved by that time. It is very important that US be the first to arrive at the market with GM fish."

China, another powerhouse of transgenic fish research, has similar plans. In 1984, Zhu Zuoyan of the Chinese Academy of Sciences' Institute of Hydrobiology in Wuhan developed the first batch of transgenic fish in the world by inserting a human growth hormone gene into the grass carp. Zhu explains the motivating factor behind the work: "In south China, it gets very cold every two years and the fish are unable to survive. But even in good weather, carp – the preferred fish – take about three years to reach market size. If we can cut that time by half, we can drastically increase catches in fish farms." Zhu has since switched from the human growth hormone gene to an "all-fish" gene construct.

Field trials show that the first generation of these modified yellow river carp - engineered with a growth hormone gene from the grass carp and a promoter from the common carp – grow 42 per cent faster than non-transgenic carp and have a better rate of absorbing food and nutrients. Like the tests at Auburn, the trials were permitted after a lengthy application process scrutinized by Chinese authorities. Says Zhu: "Tests were held at a big fish pond in Wuhan. There were special inlet and outlet barriers and the fenced off pond was protected by a guard."

Escape is a concern, says Zhu, but disagrees with Muir's Trojan gene study, asserting it is far from realistic. "We are working on a model that is more accurate than his." Zhu is especially proud of his team's method of achieving sterile fish. "Ours is a biological process, not a chemical one and it has proved very successful," he says. Pioneered a few years ago by research scientist Lu Yun, the technique involves mating a common carp with wild goldfish. The species mismatch results in triploid fish, which are then crossed with fertile transgenic fish to get completely sterile fish. "Aquabounty's method is at best 95 per cent successful, while our method is a 100 per cent success," claims Zhu.

Though transgenic fish have been around in China for two decades, there are no plans on marketing the fish at this time in either the United States or China. "In the U.S., nobody eats carp, salmon is everybody's favorite," says Zhu. And China seems to share Cuba's hesitation. "It is very difficult to commercialize transgenic fish because China is not interested in sparking a public relations disaster by becoming the first nation in the world to release transgenic fish into the market," acknowledges Zhu. The introduction of transgenic fish appears like a race where everyone wants to finish second.

The wrangle over transgenic fish is double-edged. Each camp in the controversy want to win both sides of the argument. The same environmentalists who buck biotech fish – popularly called "frankenfish – also oppose farmed fish, saying the large farms cause pollution and disease. They also call for reductions in open-sea fish trawling to preserve the environment. The industry wants to have it both ways as well: explaining that biotechnology is just accelerated evolution, yet at the same time aggressively pursuing patents and intellectual property rights that critics say, is done in a secretive manner. Aquabounty's story looks eerily similar to that of Monsanto, the giant seed company that sold high-yielding – but sterile – seeds to farmers across the developing world.

At present, no transgenic animals have been approved for human consumption, but Hallerman feels it is probably only a matter of time before transgenic fish are out in the market. "The consensus seems to be that transgenic salmon will be approved by the end of the year. There are another 30 or so GM fish awaiting approval and if Aquabounty is successful it will open the flood gates," he says. The FDA has already conducted detailed safety evaluations of the human growth hormone protein and approved its use in dairy products. The agency has also found that non-primate hormones in food are safe for human consumption. Such results will no doubt have a strong bearing on the approval of transgenic fish. Whether Aquabounty will be the first company to win approval is open to speculation, but the chances of somebody getting the green light are quite strong. The potential impact on U.S. markets is hard to judge, especially if more states and restaurants ban the product. But researchers in Cuba and China are eagerly watching. Perhaps, it is they who are awaiting FDA approval. Once transgenic fish make their way into the highways of the open ocean, there can be no recall. Then it is just a matter of waiting to see if the benefits outweigh the risks.

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