A Feast of Gene-Splicing Down on the Fish Farm

Manipulate fish embryos and you can get faster-growing carp, freeze-resistant halibut, and a sisterhood of salmon

THIS SUMMER, FOR THE FIRST TIME, FISH geneticist Rex Dunham is keeping some of his favorite carp outdoors, in ponds behind the campus of Auburn University in Auburn, Alabama. To see his pets, though, he'll have to get past surveillance cameras and the locked gates they monitor, cross a series of dikes, and untangle the fine screen that is stretched across the small, manmade water holes they swim in. These are no ordinary carp. Genetically engineered by Thomas Chen of the University of Maryland's Center for Marine Biotechnology in Baltimore to grow faster than ordinary specimens, they are the first gene-spliced fish to sojourn outside a laboratory, in "natural" conditions. Because of public concerns about the release of genetically altered life forms, Dunham and his colleagues want to make very sure that, come floods, animal predators, or human intruders, the fish stay in their ponds.

Other researchers have experimented with genetic techniques for improving the quality and yield of livestock such as pigs. But for mammals the only practical route to date has been to remove a tiny fertilized egg, alter it, and reimplant it in the uterus—a painstaking business. Fish eggs, on the other hand, are large and readily accessible—deposited by the thousands in open water. As a result, researchers are making rapid progress in tweaking the genes of salmon, trout, catfish, and other farm fish. If regulatory obstacles and public unease can be overcome, researchers hope to serve up "domesticated" fish that, thanks to genetic engineering, will reach market weight faster than natural strains and will also be hardier—more resistant to diseases and to freezing in winter.

A look at the economics of global aquaculture explains why. Raising fish in freshwater ponds and sea pens is already big business; in 1990, worldwide sales topped $22 billion, according to the international Food and Agriculture Organization (FAO), accounting for 15% of the fish consumed worldwide. And that proportion can only grow, because the "take" of wild fish has leveled off; more intensive fishing, fisheries regulators say, would cut into spawning, reducing future catches. By the turn of the century, the FAO estimates, farm-raised fish will make up 20% of world supply and will provide a prime source of protein for developing nations. The United States has a particular interest in raising the productivity of aquaculture, given its $2.4-billion trade deficit in fish last year.

One way to do so, tried in the mid-1980s, is simply to feed the fish with synthetic growth hormone. In trials by Chen, Dunham, and others, the hormone-fed fish gained weight up to twice as fast as normal, but the gains came with a crippling economic downside. Large-scale production of the purified hormone is expensive, and the fish didn't always absorb it efficiently. What's more, public wariness about milk from dairy cows injected with bovine growth hormone convinced many researchers there was no future in artificial hormone-fed fish.

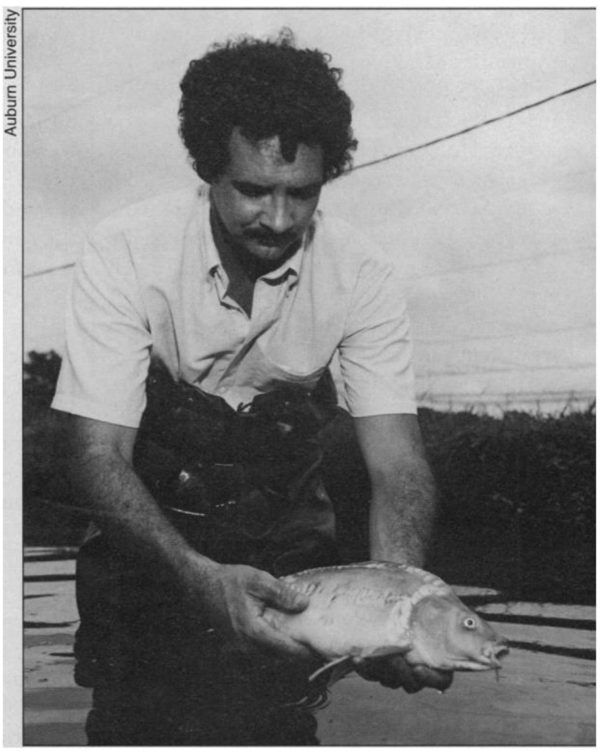
So aquaculturalists have moved to the next step: transferring into the fish cloned genes that boost the production of natural growth hormone. The initial gene transfer—generally done by microinjecting the cloned DNA into an egg—is tricky; the foreign gene doesn't always take, and many of the modified eggs don't survive. But after a successful transfer, the gene is there to stay in the fish lineage. A few hundred altered fish can spawn a few hundred thousand in several years' time, passing along all the benefits of the new gene at no added cost.

Chen's group in Baltimore pioneered the technology in carp and rainbow trout. They injected a cloned growth-hormone gene into fish embryos a few hours after fertilization. About half the embryos survived, and an average of just under half of those integrated the new gene into their own DNA. The transgenic fish and their offspring both grew from 20% to 46% faster than ordinary specimens. In the laboratory, the fish seemed robust, so Chen sent them off to Dunham, his research partner, to see how they would thrive under real aquaculture conditions—seasonal temperature changes, uneven food availability, and competition from unmodified fish.

Even if the fish fare well in the ponds at Auburn—and the outcome of Dunham's tests will be several years in coming—a number of research questions will remain before engineered fish find their way to the dinner table. Chen and other researchers are looking for techniques for boosting the efficiency with which foreign genes are integrated into the eggs' DNA, and they'd like to improve the expression of the gene to get higher growth hormone levels. That means finding better promoters—the regulatory sequences of DNA near the gene, which control its expression.

Chen and his colleagues are also trying to extend this growth-enhancing technology to some other commercial species, such as shellfish. Doing so means finding new techniques for getting foreign DNA into the embryos, Chen says; his microinjection technique is too invasive for the fragile embryos of most shellfish. He and two others—Dennis Powers of Stanford University and Koji Inoue of Nippon Suisan Kaisha, Ltd. in Tokyo—are now examining gentler means of getting foreign DNA into the embryos, among them electroporation, in which a brief electrical pulse makes the membrane of the embryo temporarily permeable.

Other researchers are taking a different tack for boosting aquaculture productivity: genetically boosting survival rather than growth. John Spence, director of the Cana-



Auburn University

Not your ordinary carp. *Rex Dunham releases a transgenic fish into a secured pond at Auburn.*

dian Aquaculture R&D Council in Vancouver, points out that fish farmers lose much of their potential revenue to diseases. Although antibiotics can sometimes control the spread of pathogenic bacteria and fungi, there are no commercially available remedies or vaccines for fish viruses. So Chen and others are dreaming of ways to engineer immunity into their fish. In one approach the fish would be equipped with a gene that codes for an antisense RNA sequence (see page 510) tailored to block the replication of the virus. An alternative disease-fighting gene would code for a viral-envelope protein, which would occupy the receptor sites on the cell surface ordinarily used by the virus, preventing it from binding and entering cells.

Cold as well as disease takes a toll on fish farms. Many halibut and Atlantic salmon raised on fish farms in Canada, for example, die when their body fluids freeze in winter. Genetic engineering has already shown promise for making crops such as tomatoes more resistant to frost, and in the hands of Garth Fletcher of the Memorial University of Newfoundland it promises to do something similar for the fish.

Fletcher and his colleagues are taking advantage of a gene from the winter flounder, a species that lives wild in Arctic waters. The flounder avoids freezing thanks to an antifreeze gene, which causes the fish's liver to produce proteins that bind to nascent ice crystals in the fish's blood, halting their growth. Fletcher and his colleagues have transferred the gene into Atlantic salmon and seen antifreeze compounds circulating in the blood of the transgenic fish. Preliminary results indicate that the engineered fish are better than their normal brethren at withstanding frigid water, according to Fletcher.

On the other side of the continent, salmon farmers are struggling with a different predicament, one that also represents an opportunity for genetic engineering. Male and female Chinook salmon, which account for most of the salmon raised on fish farms in British Columbia, grow at different rates—and the difference shows up in the quality of the fish. Males reach commercial weight in 2 to 3 years, almost twice as fast as females, but their rapid growth leaves them hook-jawed and mealy fleshed, making them less appetizing than the female fish. One solution, being tested by Robert Devlin and his co-workers at the Canadian Department of Fisheries and Oceans' laboratory in West Vancouver, is to engineer out the males. He and his colleagues are doing so by creating male brood fish that carry two X chromosomes, so that they father only females.

Chen thinks commercializing such transgenic fish—whether engineered for faster growth, slower growth, freeze resistance or viral immunity—would be fairly straightforward. The gene-altered eggs would be produced at central laboratories, then delivered to fisheries, which would raise brood stock in secured ponds on land. The brood stock's progeny would supply ponds or sea pens, where the marketable fish would be raised. Because sea pens, enclosed by nets, are less secure than ponds on land, the fry to be raised there would be sterilized—by applying heat, pressure, or chemicals to the fertilized eggs—to keep any escaped fish from mixing its engineered genes with those of its wild kin. Chen thinks the added expense of raising fish this way would be more than compensated by the gains in productivity.

Others in aquaculture are not so sanguine. "The goal of commercializing transgenic fish is fraught with practical problems," says Spence of the Canadian government. He points out that fish farming is done on slim profit margins, which might not be able to bear the cost of engineering the eggs, shipping them, and sterilizing the fry. He also foresees regulatory obstacles, which might require more elaborate schemes for preventing environmental release of the fish than Chen envisions. After all, regulations governing transgenic fish don't even exist yet in the United States or Canada. "This work won't be in routine industrial use until late in the decade," agrees Memorial University's Fletcher.

For now, researchers are cautiously testing the waters of public acceptance. The precautions in Dunham's test—a test approved by the Department of Agriculture, by the way—would make a military weapons laboratory proud. They include even more than the fences, the dikes, the screens, and the 24-hour surveillance. His ponds are rigged with a system that would release quantities of poison, killing everything in sight, if the waters ever rose in a flood. Such are the trials of the genetic engineer. In time, though, Dunham's poison may become everyone's meat—or fish. ■ MARK FISCHETTI

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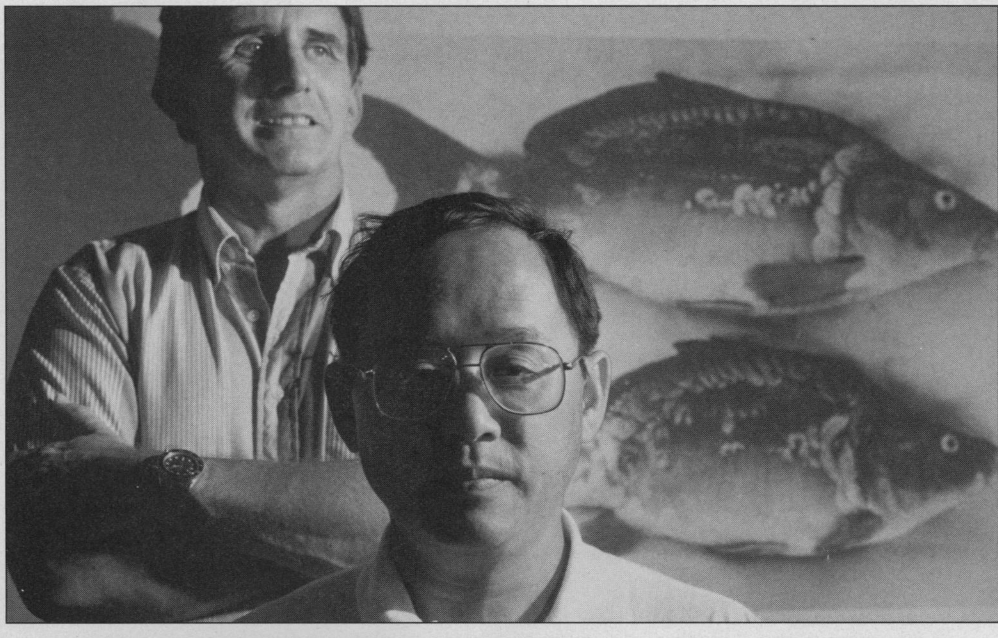

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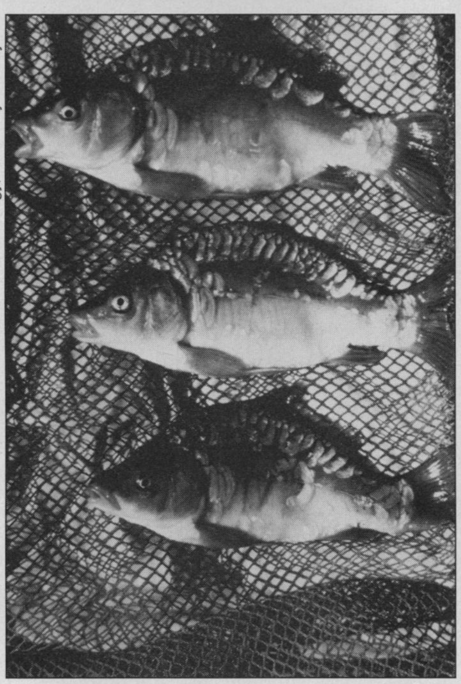

Center for Marine Biotechnology, University of Maryland

The big fish. *Thomas Chen* (foreground) *and Dennis Powers. At left, two transgenic carp and a normal sibling* (bottom) *without the growth-boosting gene.*