RELEASING ENGINEERED FISH

TRANSPLANTED FISH CAST SHADOW

WASHINGTON, D.C.—For more than a century, foreign and domestic varieties of fish have been transplanted willy-nilly from one environment to another across the U.S., sometimes with horrendous consequences. The zebra mussel's introduction into the Great Lakes has proved enormously damaging to this complex habitat. Because the mussels actively colonize metal surfaces, they clog water intake and treatment systems, affecting large-scale industrial and urban systems.

With that history in mind, experts are debating whether such experiences provide a suitable model for evaluating genetically engineered fish before they are released into open environments. At the very least, any planned introductions of engineered fish need to be assessed "very carefully," say experts.

Exotic, but genetically unengineered, fish were the principal objects of a workshop, "Native Fish, Introduced Fish: Genetic Implications," sponsored by the National Audubon Society (Washington, DC) and held here recently. But concerns about the potential impact of new genetically engineered varieties of fish lurked in the minds of the participants from academic institutions and state and federal agencies, as well as the environmental community.

Although perhaps a dozen U.S. laboratories are developing genetically engineered fish, so far only Rex Dunham's research group at Auburn University (Auburn, AL) has sought federal approval for conducting outdoor studies (*Bio/Technology* **10**:492, May '92). Dunham voluntarily submitted his proposal to study catfish engineered with rainbow trout growth-hormone genes to the U.S. Department of Agriculture (USDA, Washington, DC). Despite a lukewarm approval, last summer USDA officials told Dunham he could go ahead.

Early research

Much of the early research effort to apply genetic engineering technology to fish has concentrated on moving hormone genes from one species to another as a means of enhancing the rate and efficiency of growth in the recipients of the transferred genes. Even though recent reports indicate that such genetic manipulations can significantly enhance growth rates, there is still no definitive information as to how efficiently engineered fish convert food into body weight, notes Anne Kapuscinski of the University of Minnesota College of Natural Resources (St. Paul, MN).

Other early genetic engineering efforts in fish include the transfer of genes that produce antifreeze glycoproteins, with the aim of making the recipient fish able to withstand colder temperatures, thereby potentially enlarging their habitats. Moreover, through comparative studies, molecular biologists are finding that mammalian and fish gene structures are more closely homologous than was once thought, suggesting that far more numerous and more broadly divergent gene transfers may prove possible.

The first significant wave of intentional introductions of fish species into the U.S. from Europe and Asia began about a century ago, and they have continued into the present. Much of this effort is undertaken to support the domestic aquaculture industry, which has enjoyed high growth during recent years. Cultivation of channel catfish accounts for about half of this growth.

Growth of the aquaculture industry is being driven by several forces. For one, the general population is consuming more fish and seafood. For another, sports fishing is growing at a 15-20 percent annual rate, putting pressure on

"A QUANTUM LEAP"

MYCOGEN WINS ONE, LOSES ONE

SANTA CRUZ, Calif.—What's the story with Mycogen (San Diego, CA)? In the span of less than a week in August, the biopesticide company surrendered some of its basic patents on *Bacillus thuringiensis (B.t.)*-derived biotoxins to Novo Nordisk (Bagsvaerd, Denmark) and picked up a major new agricultural company—Lubrizol's (Wickliffe, OH) seed-and-plant division Agrigenetics. The events are unrelated, but they mark the transition of a biotech niche player into a market force. Of the two, J. Jeffrey Cianci, managing director at Bear Stearns & Co. (New York), ranks the Agrigenetics acquisition as "95 percent of the importance. That's the company maker."

In this deal, which was first announced last April, Mycogen paid $135 million for Agrigenetics. The two companies will jointly develop genetically engineered pest-resistant crop varieties. Mycogen will have a 51-percent stake in the venture; it will transfer 2.29 million common shares and issue $39.4 million worth of new preferred stock to Lubrizol. Lubrizol thus increases its stake in Mycogen to 25 percent. Once the preferred stock is converted, this will jump to 33 percent. Analyst George S. Dahlman, managing director at Piper, Jaffray & Hopwood (Minneapolis, MN), applauds the acquisition. "This brings Mycogen into a full-blown business that has a great future. That is, a seed business packaged together with a higher-powered biotech program." Cianci agrees, saying that "it represents a quantum leap for Mycogen. It's what it took for Mycogen to become big time."

With the acquisition, Mycogen also expands its technology base and acquires patents related to plant gene-transfer technology, including those for *B.t.* toxins. This may offset the company's recent loss of other *B.t.*-related patents to Entotech (Davis, CA), a division of Novo-Nordisk. The two companies settled patent lawsuits dating from 1988, in which Entotech charged Mycogen with both infringement and interference. At the crux of the issue is a matter of identity: it turns out that Mycogen's strain *B.t. san diego* and Entotech's *B.t. tenebrionis*, whose discovery predates *B.t. san diego*, are the same, at least for patent purposes. According to the joint press release: "Mycogen has conceded that the original *B.t. san diego* is *B.t. tenebrionis*, and has therefore disclaimed several Mycogen patents directed to the use of Coleoptera-specific *B.t.* Moreover, other Mycogen patents in this area have been assigned to Entotech." Mycogen paid Entotech $1.3 million to resolve the claims, and another $3 million for a non-exclusive, lifetime license to the patents.

Novo Nordisk claims that it is now the "sole owner worldwide of all basic patent rights covering *B.t.* toxins effective against beetles." Entotech's president Pam Marrone says that Entotech's patents were "for any strains of *B.t.* that were Coleoptera-active. Mycogen had the actual DNA sequence for *B.t. tenebrionis*, which postdated ours. Therefore the DNA patent becomes ours. So do several others relating to specific Coleopteran pests such as bollweevil and elm leaf beetle."

But Mycogen's executive vice president Andrew Barnes counters that Mycogen's patents "all related to a specific type of *B.t.* that produces a certain toxin of a certain weight range for certain Coleoptera. We know that the toxin is active

hatcheries to restock freshwater rivers and streams in which native species are overfished. Pressure is also increasing on saltwater resources, where both commercial and sports fishing activities have intensified.

Genetic diversity

In part because of this fast growth, there is increased "concern" about the decline in genetic diversity among the principal species of fish raised by aquaculture, says Meryl Broussard of USDA's Cooperative State Research Service. Experts are "not sure if some domestic lines could survive," he says, explaining that if a new disease were to arise, it might decimate a genetically homogeneous stock of a particular species. Although producers are coming to recognize this risk, market pressures have tended to make them put off dealing with it.

The recognition of such problems is leading to changes in attitude among members of the industry, including those serving the sports fishing segment, according to Reggie Harrell of the University of Maryland's Horn Point Environmental Laboratory (Horn Point, MD). For instance, some fisheries managers see their role as "restoring and enhancing" the quality of fish available in streams or lakes, not just restocking to assure that everyone who fishes can maximize their daily catch, says Harrell.

Transplanting fish from one environment to another has often proved damaging. Will releasing engineered fish prove equally damaging?

Not all fish introductions are carried out by professionals or according to careful planning, however. Indeed, many introductions are haphazard or accidental. Aquarium shop owners and fish hobbyists introduce numerous exotic species of fish into various habitats in arbitrary fashion. Various species, including the infamous zebra mussel, survive intercontinental journeys in ship ballast waters, which are sometimes referred to as global "conveyer belts" for aquatic species.

In the aggregate, this combination of deliberate efforts and accidental introductions of fish species to the North American continent has led to some 70 species of exotic fish becoming established in U.S. waters, accounting for 10 percent of overall fish species. In addition, more than 160 transplanted native species of fish have become established within the U.S. in novel habitats.

Environmental impact

The environmental impact of fish-species transfers can be measured on several levels and virtually always includes long-term effects on biodiversity. In a specific habitat, an introduced species of fish competes for limited resources, such as space and nutrients. A newcomer species may also bring parasites and other disease-causing microorganisms with it, sometimes leading to the disappearance of resident species of fish and plants.

Consider the rud, a small fish first brought from Europe to the U.S. about a century ago that, more recently, was introduced as a bait fish into Lake Oconomowoc in Wisconsin. Some time after that introduction, the rud seemed to disappear, but fish geneticists soon realized that it was forming inter-generic hybrids with native golden shiners.

Damage from non-native fish introductions led to passage by Congress in 1990 of the Nonindigenous Aquatic Nuisance Prevention and Control Act. The act, though, is limited in scope, and subsequent efforts to develop a more encompassing federal policy governing the introduction of aquatic species have failed. Moreover, efforts at the state government level have been minimal and inconsistent from one state to another. "A lot of people think there should be a federal policy and uniform rules, not just a free-for-all," says Maureen Hinkle, Audubon's director of agricultural policy.

With the prospect looming for outdoor tests of genetically engineered fish, such as those planned by Auburn's Dunham, it makes sense to begin "early on to do risk assessments," says the University of Minnesota's Kapuscinski. However, she says, such studies are currently "not a high priority. Most research goals are driven by the desire to build better fish for aquaculture."

Some observers argue that modifying one or a few genes in a particular species of fish will be of little environmental consequence. Kapuscinski disagrees, referring to a white paper issued in 1989 by a group of scientists from the Ecological Society of America (Bethesda, MD). In addressing the general issue of gene transfers between species—not solely fish—they concluded that "novel combinations of traits are likely to play novel ecological roles." For instance, incorporating traits into a fish that would enable it to extend greatly its natural habitat—into both sea water and fresh water or into extremely cold rather than just temperate habitats—could have enormous ecological impact.

Currently, none of the researchers genetically engineering fish plan to introduce them into open waters. Rather, they are considering testing them in indoor hatcheries and in outdoor facilities. Yet judging from experiences with non-native and unengineered species of fish, once an organism is out in the open environment, it can become difficult to control and probably impossible to eradicate. "We might need to teach the public that introductions are forever," says Ellen Marsden, director of the Lake Michigan Biological Station (Zion, IL).

—**Jeffrey L. Fox**

only on a few Coleoptera—Colorado potato beetle, elm leaf beetle—but it doesn't work on many others."

Barnes says that the patents actually only cover one of Mycogen's many products, M-Trak. It incorporates *B.t.* toxin encapsulated by killed *Pseudomonas fluorescens* bacteria and is effective against Colorado potato beetle. Mycogen's pesticidal arsenal is much more diverse: in fact, the company has collected and screened thousands of *B.t.* strains for novel pesticidal activities.

The $4.3 million settlement might have put a crinkle in Mycogen's financial sheets, though the company had $70 million in the bank prior to it. "But the settlement allows us to continue on with our business plans uninhibited," says Barnes. "The Agrigenetics announcement is just evidence that we've got other business to get to." Analyst Dahlman concurs that the settlement "had no impact on the business. Essentially they are where they were before—they can sell *B.t. san diego*."

—**Jennifer Van Brunt**