RD: ... For those of you who were at the session Wednesday evening, you heard Anne Kapuscinski speak about engineered fish populations. And our speaker, who s going to be up here in a second, Eric Hallerman, is a close associate with Anne. He is now at The Department of Fisheries and Wildlife Sciences at Virginia Polytechnic Institute and State University. And in the conversation I had with him just before the talk, he was very pleased with that session Wednesday evening. Not necessarily because of what was said, but because Anne set him up very nicely. I think he s going to elaborate on a lot of the things that Anne spoke about Wednesday evening. And I m just kind of stalling here, until this thing boots up. . . .

Let me introduce Eric Hallerman. And he s going to speak to us now about Ecological and Evolutionary Issues Posed by Genetically Modified Fishes. Eric?

ECOLOGICAL AND EVOLUTIONARY ISSUES POSED BY GENETICALLY MODIFIED FISHES

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EH: Well, thank you, Rob, and good morning, everybody. Gene transfer is a key biotechnology for manipulation of animal genomes. By introducing a gene whose expression is under novel regulation, biotechnologists can achieve dramatic impact upon valued phenotypes. For example, I show here on the left a transgenic Atlantic salmon expressing an introduced growth-hormone gene. It grows four to six times faster than nontransgenic siblings, accompanied with a 20% improvement in feed-conversion efficiency. With the prospect of improved production efficiency, it s not surprising that some aquaculturists want to go ahead and raise transgenic fish and shellfish commercially.

Commercialization of transgenic fish and shellfish is controversial for a number of reasons, including concerns about possible ecological and evolutionary impacts. In this morning s talk I ll explore the possible impacts of transgenic fish and shellfish on conservation of native aquatic biodiversity.

To provide the technical context, I ll start out by briefly describing the rationale for producing transgenic fish and shellfish, and describe the status of the commercialization effort. I ll focus most of my talk on ecological and evolutionary concerns posed by commercial production of transgenic fish and shellfish. And to close, I ll suggest approaches for managing the ecological and evolutionary risks posed by commercial production of transgenic fish and shellfish.

Development of aquatic GMO s poses benefits to commercial aquaculture. Gene-transfer technology has been applied to approach several breeding objectives. For example, growth-hormone genes have been introduced in over a dozen species to increase growth rate. Several experiments have attempted to increase the freeze resistance of freeze-sensitive species by introducing antifreeze polypeptide genes from Arctic or Antarctic fishes. Although significant freezing-point depression has yet to be demonstrated.

Improvement of other economically important traits will depend on identification of key genes. For example, should genes concerning resistance to key diseases be identified, they could be transferred into susceptible species or strains. If a key biochemical pathway has been disrupted by a lack of one enzyme, the function of that pathway can be restored by expression of a gene for that one enzyme. Such pathways might include those for synthesizing Vitamin C or omega-3 fatty acids, now required [nutrients for feed] for many fishes. Development of transgenic lines of over 20 species is pursued by at least 20 research groups in over a dozen countries.

Efforts to commercialize transgenic fish lines are ongoing. I show here an advertisement placed in a trade journal by AF Protein, the producers of the transgenic salmon that I showed in the first slide. It was mentioned in the opening session Wednesday night. AF Protein has petitioned the Food and Drug Administration for approval to market eggs for transgenic Atlantic salmon to commercial salmon growers. They also have transgenic lines of rainbow trout.

Rex Dunham of Auburn University reportedly has started a process of seeking FDA approval to market his transgenic channel catfish. Others may also have applied to FDA. But, as Anne noted Wednesday night, applications remain confidential, unless the applicants choose to publish them. Transgenic carp and tilapia are reportedly already in commercial production in China, and transgenic tilapia in Cuba, although I have no data suggesting the scale or conditions of their production.

What about commercial production? Should we be concerned? Commercial aquaculture operations have routine, often significant, escape of fish. Escapes can occur through equipment failures, during handling of transport operations, through predator intrusion into facilities, as a result of storms, or by other mechanisms. In particular, escapes of salmon and trout from pen facilities, such as this one, are common. And range from minor incidents, where a few fish escape, to massive escapes of tens or even hundreds of thousands of fish.

Although salmon-farm operators are attempting to prevent escapes by upgrading confinement systems, installing predator-deterrent devices, and by taking other actions, we still must assume that escapes will occur. Because of the possibility of escapes of aquatic GMO s from commercial-production facilities to natural ecosystems, we must consider not only their benefits to aquaculture, but also any environmental hazards that they might pose.

We can anticipate ecological hazards to a range of species with which a GMO interacts in the accessible ecosystem, and genetic hazards to conspecific natural populations. Ecological hazards include the possibility of heightened predation or competition; colonization of GMO s in ecosystems outside their native range; and, possibly, alteration of population or community dynamics due to the activities of the GMO.

Fertile GMO s could interbreed with natural populations. Any genetic or evolutionary impact will depend on the fitness of novel genotypes in the wild. We have to consider cases where fitness relative to the wild type is high, posing intergression of transgenes into natural populations and possible fixation.

We also have to consider the opposite case, where maladaptive traits might be introduced into native populations, posing a hazard to the viability of the receiving population. Risks posed by a given commercial application of transgenic fish or shellfish must be assessed on a case-by-case basis considering not only the species in the transgene, but also the particular transgenic line and the receiving ecosystem at issue.

This is because each transgenic line is different. Expression of the transgene will depend not only on the structural gene, but also on the regulatory element controlling the expression of that transgene and the genomic side of integration. The receiving ecosystem is the context in which we have to consider the viability and fitness of transgenic genotypes. With these caveats, what I ll do through the bulk of my talk this morning is consider the experimental data concerning ecological and evolutionary hazards posed by transgenic fish.

Some of the most useful data for exploring the possible ecological and evolutionary impact mechanisms, and quantifying the associated risks, were collected using a transgenic-model species. Muir and Howard, and their associates, obtained life-history data on a small cyprinidontoid fish, the Japanese medaka, that I show here. The line they used expressed a gene construct that incorporated an Atlantic salmon growth-hormone promoter fused to the human growth-hormone gene.

The deterministic model that they developed, using the data that I ll describe, predicted that, under certain conditions, a transgene introduced into a natural population by a small number of transgenic fish would spread as a result of the enhanced mating advantage enjoyed by the larger individuals. But the reduced viability of the offspring could, under certain conditions, cause local extinction of the population.

This model became termed the Trojan gene hypothesis. The predicted time to extinction of a wild-type medaka population would be a function of the mating advantage of the transgenic males relative to the wild-type males, and the relative viability of transgenic offspring.

Muir and Howard subsequently elaborated their approach for evaluating risk, putting estimates of fitness parameters from a founder population of medaka into a recurrent model to predict changes in transgene frequency after a simulated transgenic release. Aspects of an organism s life cycle are grouped into net fitness components regarding juvenile viability; adult viability; age at sexual maturity; female fecundity; male fertility; and mating success. They generalized their model s predictions by using various combinations of fitness-component values, in addition to the empirically derived estimates of those values for medaka.

For a wide range of parameter values, the model predicted that transgenes could spread through populations, despite high juvenile-viability costs, if there were sufficiently high positive effects on other fitness components. Sensitivity analyses showed that transgene effects on age at sexual maturity should have the greatest effect on transgene frequency. Followed by juvenile viability, mating advantage, female fecundity and male fertility, with the least impact due to changes in adult viability. If there s any one paper that I would have you read as a result of hearing my talk this morning, this would be the one. The authors are Muir and Howard, and it ll be in *American Naturalist* any time now it s in press.

Most risk-assessment studies involve aquaculture species, using transgenic lines under development for possible commercial use. Several studies have focused on Atlantic salmon, specifically on transgenic lines developed by Aqua Bounty Farms, the subsidiary of AF Protein, that expressed and introduced ocean pout antifreeze polypeptide promoter fused to the Atlantic salmon growth-hormone gene. As I mentioned at the outset, some of these lines grow four to six times faster than nontransgenics, with improvements in feed conversion efficiency.

To support their rapid growth, the transgenic salmon consume food at a more rapid rate than control salmon. Their oxygen uptake is about 60% more than controls during routine activity and during sustained swimming. The gill-surface area available for respiratory exchange in the transgenic salmon was 1.24 times that of the control salmon, which did not parallel the 1.6 elevation in oxygen uptake. The metabolic cost for a 1 kilogram salmon to swim 1 kilometer was 1.4 times that of control salmon.

Abrahams and Sutterlin tested the hypothesis that the high rate of growth of transgenics should be correlated to willingness to increase exposure to a predator. They observed the behavior of size-matched transgenic and control salmon in experiments where fish could feed in safety or in the presence of a predator. The growth-enhanced transgenic fish were significantly more willing to risk exposure to a predator in order to gain access to food and also exhibited increased feeding rate and average speed of movement.

However, transgenic fish did reduce their exposure to predators in response to increases in the magnitude of risk, suggesting that their more active behavior might not necessarily translate into increased susceptibility to predation. The transgene salmon did lose their parr marks and turn silvery sooner than nontransgenics. That increased their visibility, and, hence, their vulnerability to predation.

Considering all of these findings together, the authors, who worked for the company that s producing these fish for possible commercial use, pointed out that it s difficult to assess the likely outcome should transgenic fish escape into the wild and invade wild populations.

Assume you re looking at a brown trout. (Laughter) Nontransgenic fish injected with a grown-hormone protein provide a ready model for transgenic fish expressing introduced growth-hormone genes. Among studies of GH-injected fish are several on brown trout, a congener of Atlantic salmon. Brown trout injected with GH were more willing to risk exposure to a predator than noninjected fish. GH treatment had no effect on dominance relationships or on the quantity of ingested food. Mortality of brown trout in a near natural stream was not increased by GH treatment, although lipid reserves were lower in the GH-treated fish.

GH-treated fish did grow faster than controls. The growth-promoting effect of the GH was much more pronounced in the hatchery than in the wild, suggesting that the payoff associated with the increased growth investment is higher under hatchery conditions, where there s an unrestricted food supply, than the wild, where food availability is limited. The authors concluded that fitness of GH-treated and control brown trout were similar and, hence, escaped growth-hormone-manipulated fish may compete successfully with wild fish.

The Pacific salmonids include a number of aquaculturally important species, including coho salmon, which you see here, and rainbow trout. And they ve been the subject of a large number of transgenesis experiments, including risk-assessment experiments.

Coho salmon, expressing a salmon metallothionein B promoter growth hormone 1 construct, exhibited extraordinary growth. Shown above, on average 11 times that of nontransgenic full sibs. Transgenic salmon underwent precocious transformation to the smolt stage during their first fall, approximately six months before their nontransgene siblings. At just two years of age, five males became sexually mature, and four transmitted the transgene in their sperm.

Should a transgene be transmitted to a wild population of coho salmon, what risks might be posed? Because the absolute swimming speed of fish increases with body length, Ferrell et al. tested the hypothesis that growth-stimulated transgenic fish would prove faster swimmers. That s not what they found.

Here we have the length of the fish and the critical swimming speed the maximum swimming speed that the fish can sustain for a given period of time. Here s the critical swimming speed for transgenic fish, which compares with that of much smaller nontransgenic controls. The critical swimming speed of the transgenics is much less than that of the size-matched nontransgenic controls.

The authors hypothesized that poorer swimming performance of the transgenics arose from a developmental delay, or from disruption of locomotor muscles or some sort of associated support systems, such as respiratory, circulatory or nervous systems. Some growth-enhanced fish exhibited abnormalities of opercular of gill-cover morphology that might disrupt respiration and contribute to poor swimming performance.

Collectively, these observations raise the possibility that the heightened ability of transgenic coho salmon in some terms of some physiological process, such as growth is gained at the expense of the locomotor system. And, perhaps, other systems. An inference that may be pertinent when you re estimating the risks associated with the escape of transgenic salmon and their interbreeding with wild populations.

In feeding trials, Devlin et al. compared the intake of contested feed pellets by size-match pairs of one control and one transgenic fish. For the first three pellets of each feeding trial, the transgenics consumed 2.5 times more contested pellets than controls, supporting the hypothesis that GH transgenesis increases the ability to compete for food.

Overall, transgenic fish consume 2.9 times more pellets than nontransgenic controls, indicating a high feeding motivation of transgenics relative to nontransgenics. The authors concluded that, depending on how transgenic and wild individuals differ in other fitness-related characters, escaped transgenic fish may compete successfully with native fish in the wild.

What would be the outcome of intergression of transgenes into wild populations? I think this is the most interesting study that I ll cite among the group. Devlin et al. examined the growth enhancement due to expression of a growth-hormone gene in both wild and selectively bred rainbow-trout strains. Let s look at panel B first.

This shows the size at maturity for the respective strains. A cultured transgenic female, cultured transgenic male, wild female, wild male. In panel A we see the effect of the transgene in the respective stocks. A domestic stock its final size was not dramatically increased by expression of an introduced gene. The wild stock its size at maturity was dramatically affected by expression of a transgene. I also find it interesting that the slender body morphology of this stream population of salmonids is retained. Look how much more streamlined this fish is than the selectively bred fish.

Panel C shows the effect of interperitoneal injection of growth hormone into their respective strains. The upper two lines are the commercially produced, selectively bred strain, and the difference between the upper two lines is not dramatic. The lower two lines show the growth enhancement of growth hormone in the wild strain. The wild strain showed a dramatic response to the injection of growth hormone.

Panel D shows the effect of the injection of growth hormone phenotypically. The cultured stock showed an increase in size at maturation, but it wasn t particularly dramatic. The wild stock showed a very dramatic enhancement of age at maturity due to injection of growth-hormone protein.

The last panel is the most interesting one. Here we see the size, at a particular age, of nontransgenic fishes, including a wild stock; the F-77, which is another wild stock; and a domesticated stock. Here we see the size of the F-77 stock, once it s been made a transgenic, and then the size of a hybrid of the domestic by the F-77 line. Far and away, the highest weight was shown by the hybrid transgenics. Both the domestic and the wild strain transgenic trout had reduced viability. In the case of a domestic strain, all transgenic individuals died before sexual maturation.

The key point that I m making by all of this is the effects of an introduced growth-hormone gene may be different for fishes of different background in this case, domesticated and wild stocks. The effects may depend, in part, on the degree to which earlier enhancement had been achieved by selective breeding. The key point I want you to take away from this, though, is that hybrid wild by transgenic individuals may exhibit dramatically modified phenotypes.

There are a number of studies that examine the physiology behavior of nontransgenic rainbow trout that were injected with the growth-hormone protein. Nontransgene rainbow trout treated with GH were more willing to risk exposure to a predator than uninjected fish. To clarify the role of growth hormone in social interactions, Johnson et al. observed antagonistic interactions in pairs of juvenile rainbow trout consisting of two control fish; a control and a transgenic; a control and an injected fish; or two injected fish. Aggression was lowest in the control pairs, intermediate in the control-injected pairs, and highest in the growth-hormone-injected pairs. This finding supports the hypothesis that growth hormone increases aggression levels.

Paralleling the results of the transgenesis experiment that I just showed you, the specific growth rates of the wild rainbow trout that were administered the growth-hormone gene were accelerated 2.7-fold, while those of the domesticated trout just by a mere 9%. Cranial abnormalities in silver-body coloration were observed only in growth-hormone-treated individuals of the domestic strain.

Channel catfish is the most important aquaculture species in the United States. A transgenesis experiment by Rex Dunham shown here at the left, of Auburn University aimed to accelerate the growth rate of channel catfish through expression of constructs using the long-term, or repeat element, of the *Rous sarcoma* virus, to derive expression of rainbow trout of chinook salmon growth-hormone genes. Growth acceleration of 30% was shown in some transgenic lines.

Dunham et al. showed that transgenic and nontransgenic channel catfish exhibited a similar degree of predator avoidance by a largemouth bass or a green sunfish. In unfed ponds, growth was equal, but survival less, for transgenic than for nontransgenic genotypes. In a competitive-breeding situation, mating was random among transgenic and control individuals. Among males, more transgenics than nontransgenes gave matings. The opposite was the case among females. Hence, should there be an escape, the transgene would be likely to enter, and persist, in wild populations of channel catfish.

Although not cultured in the United States, common carp is an important aquaculture species. Especially in Asia also in Europe and the Middle East. Among transgenic common carp at Auburn University, lower than expected inheritance rates for the transgene suggested either differential mortality or unstable genomic integration. However, from 30 grams onward, transgenic carp exhibited equal or higher survival rates than control carp. Under dissolved oxygen channels, the transgenic individuals lived longer before they died.

Tilapias are native to Africa and the Middle East, and have been widely introduced because of their hardiness and their ease of culture. Tilapia production is important, especially in many developing tropical countries. Martinez et al. reported growth enhancement in transgenic hybrid tilapia, expressing a tilapia growth-hormone gene driven by the human cytomegalovirus promotor.

An aquaria transgenic tilapia showed higher feeding motivation and dominant status than nontransgenic siblings, but less than tilapia, themselves, collected from the wild. After exposure to half-strength seawater, plasma-sodium levels did not increase significantly in transgenic tilapia, but increased in nontransgenics and in wild types suggesting greater osmoregulatory ability in the growth-hormone transgenics.

Significant technical challenges were overcome to produce transgenic crustaceans and mollusks. To my knowledge, no research has assessed the ecological risk posed by culture of these organisms. I ll also add that confinement of these organisms is particularly difficult, especially at the earliest life stages.

To summarize, it s clear that our empirical knowledge of hazards posed by production of transgenic fish, and their associated risks, is limited. It appears likely that some transgenic aquatic species will pose ecological or genetic hazards should they escape from confinement and enter natural aquatic ecosystems. Many critical experiments aimed at estimating fitness parameters are yet to be conducted.

In no case do we have sufficient data to parameterize a fitness model, such as the extended Trojan gene model, and to project whether a transgene might become permanently intergressed into a natural population. We are nowhere near being able to predict the evolutionary consequences of intergression of transgenes on the evolutionary trajectory of a population or a species.

Knowledge of ecological and genetic hazards, and the associated risks posed by commercial-scale application of aquaculture biotechnology, is quite limited, given the range of decisions to be faced regarding whether to go forward with production of aquatic GMO s, in an ever-widening range of culture systems and ecological contexts.

Although cases posing serious hazard may prove to be the exception, the potential for hazard argues for careful risk assessment and risk management, combined with caution of use of aquatic GMO s in the environment. Managing risks posed by commercial production should be considered at two scales: at a particular production site and at the landscape level.

Commercial aquaculturists considering the possibility of producing aquatic GMO s at their particular operation will need to implement a suite of measures to achieve effective confinement of their production stock. The issue of how to achieve this level of confinement arose in the context of how to safely conduct experiments and watch the development of the performance standards for safely conducting research with genetically modified fish and shellfish. And I give the URL there.

Returning to the pending application for FDA approval for marketing of AF Protein s transgenic salmon, what confinement measures are under consideration? To achieve effective confinement, it ll be preferable, from an environmental-protection standpoint, to produce the salmon in indoor recirculating aquaculture systems. The production efficiencies offered by rapid grown and approved feed conversion of the transgene lines might make such operations economically competitive. But the salmon-production center is dominated by floating net-pen systems, and most potential buyers are net-pen operators.

Net pens do not provide consistently reliable physical confinement of cultured stocks hence, reproductive confinement is needed. To achieve reproductive confinement, AF Protein intends to produce only all-female triploid production stocks. Since commercial productions involve hundreds of thousands of fish, 100% triploid induction will have to be reliably achieved.

AF Protein claims to have achieved 100% triploidy among 12 families of Atlantic salmon, involving thousands of fish. At a commercial scale, with hundreds of thousands of fish, repeatable induction of 100% triploidy poses a considerable technical challenge. Assuming that 100% triploidy induction can be achieved at that scale, direct evolutionary hazards are addressed. But ecological hazards are still posed, particularly due to competitive interactions of escaped transgenics with conspecific populations.

For example, transgenic Atlantic salmon escaping from production facilities in Maine, or the Maritime Provinces of Canada, might compete with wild stocks native to several rivers in Maine, that recently were listed as endangered under the Endangered Species Act. Any heightened competition would pose a further demographic stress to recovery of those populations. In this country we at least have some regulatory oversight. Interest in production of transgenics is being considered in countries that don t have oversight policies.

Kapuscinski, Nega and I posed a regime for how to adaptively manage genetically modified aquatic organisms. I m running out of time here, but there is a reference for this in your program, under my abstract and bio. And any of you that are interested in it, we can put reprints in the mail to you.

Successful commercialization of transgenic fish and shellfish will require public confidence that food safety and environmental risks have been thoroughly addressed.

Okay. I ll assume that you caught my key points.... I d like to acknowledge the organizers of this event. I didn t mean anything by leaving off The Wildlife Conservation Society. My mistake, entirely. I d also like to acknowledge the agencies that have supported my work.

Thank you very much.

(Applause)