Assessment of Environmental and Indirect Human Health Effects of Genetically Modified Aquatic Organisms:

Proceedings of the Fisheries and Oceans Canada and Health Canada Experts Meeting, March 30 - 31, 2004, Vancouver, British Columbia

Editor

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Edited by

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ABSTRACT

Although genetically-modified fish have been produced since the mid 1980s, considerable debate remains on the potential benefits and risks that this technology may afford. The manuscripts within these proceedings reflect current views of leading scientists involved in generating transgenic fish for potential use in aquaculture and those involved in risk assessment research and regulation, as well as views on government, societal and academic policy. The manuscripts cover basic characteristics of transgenic fish, as well as their physiology, genetics, and behaviour. The major species considered for aquaculture are considered, including tilapia, catfish, carp, and salmonids, and perspectives from Europe, Asia, and the Americas are represented. In addition, significant material is presented discussing ecological risk assessment of transgenic fish. Both empirical and theoretical approaches to risk assessment are discussed, as well as limitations to current risk assessment science. Discussions following each manuscript are reported, as well as four general discussion sessions on the Canadian regulatory system, genetic containment, risk assessment methodology, and the future uses and benefits of transgenic fish technology.

RÉSUMÉ

Bien que des poissons génétiquement modifiés soient produits depuis le milieu des années 1980, les avantages et les risques possibles de cette technologie suscitent encore un débat considérable. Les manuscrits présentés dans ces actes reflètent les points de vue actuels de chercheurs réputés qui participent à la production de poissons transgéniques en vue d'utilisations en aquaculture ainsi que de chercheurs qui jouent un rôle dans les recherches axées sur l'évaluation des risques et les activités de réglementation, de même que des points de vue relatifs aux politiques gouvernementales, sociétales et académiques. Les manuscrits portent sur les caractéristiques de base des poissons transgéniques, ainsi que sur leur physiologie, leur génétique et leur comportement. Sont présentés les principales espèces considérées aux fins d'aquaculture, y compris le tilapia, le poisson-chat, la carpe commune et les salmonidés, des points de vue de chercheurs européens, asiatiques et américains et des informations importantes sur l'évaluation des risques que suscitent les poissons transgéniques pour l'environnement. Des approches empiriques et théoriques de l'évaluation des risques font l'objet d'une discussion, de même que les limites des données scientifiques actuelles aux fins d'évaluation des risques. Est également présenté un compte rendu des discussions menées à la suite de chaque manuscrit ainsi que de quatre séances de discussion générales sur le système de réglementation canadien, le confinement génétique, la méthode d'évaluation des risques et les utilisations et avantages futurs de la technologie de la transgénèse.

PROGRAM

Experts Meeting on Assessment of Environmental and Indirect Human Health Effects of Genetically Modified Aquatic Organisms

March 30-31, 2004

Crowne Plaza Hotel Georgia
801 West Georgia Street, Vancouver, British Columbia

March 30, 2004

09:00	Welcome/Opening Remarks	Peggy Tsang
09:15	Session 1 – Chair, Bob Devlin	
09:20 – 09:40	Perspective of genetically modified (GM) fish use and regulation in China	Zuoyan Zhu
09:40 – 09:50	Questions/Answers	
09:50 – 10:10	Transgenic modifications and other biotechnology approaches to strain enhancement for aquaculture	Rex Dunham
10:10 – 10:20	Questions/Answers	
10:20 – 10:40	Break	
10:40 – 11:00	The science and business of transgenic salmon: Promises and problems.	Garth Fletcher
11:00 – 11:10	Questions/Answers	
11:10 – 11:30	European perspective of GM fish use and regulation	Norman Maclean
11:30 – 11:40	Questions/Answers	
11:40 – 12:00	Genetic modified fish research in Cuba	Rebeca Martinez
12:00 – 12:10	Questions/Answers	

12:10 – 13:15	Lunch	
13:30	Session 2 – Chair, Tillmann Benfey	
13:30 – 13:50	Academic uses of GM fish technology: A call for a common sense approach to regulation	James Wright
13:50 – 14:00	Questions/Answers	
14:00 – 14:20	Variables influencing risk assessment data derived from laboratory-contained GH transgenic coho salmon	Robert Devlin
14:20 – 14:30	Questions/Answers	
14:30 – 14:50	Application of modelling to risk assessment of GM fish: The net fitness appoach	William Muir
14:50 – 15:00	Questions/Answers	
15:00 – 15:20	Break	
15:20 – 15:40	Use of nontransgenic approaches for risk assessment	Jorgen Johnsson
15:40 – 15:50	Questions/Answers	
15:50 – 16:10	Status of environmental biosafety science on genetically engineered fish and policy implications	Anne Kapuscinski
16:10 – 16:30	Questions/Answers	
16:30 – 16:50	Social/environmental issues regarding genetically modified fish use: Policy considerations	Dennis Kelso
16:50 – 17:00	Proposed Canadian initiative of EENLO research network	Stuart Lee
17:00 – 17:15	Questions/Answers	

March 31, 2004

09:00 - 09:15	Opening comments	Peggy Tsang
09:15 – 10:00	Overview and discussion of regulatory processes for transgenic fish in Canada	George Arvantakis
10:00 - 10:45	Current and future approaches to genetic containment.	Tillmann Benfey
10:45 - 11:00	Break	
11:00 - 11:30	What approaches are suitable for risk assessment of GM fish? What is the best way to deal with uncertainty in data for risk assessments?	Bill Muir
11:30 - 12:00	What kinds of traits are likely to be modified in the future? What are the benefits (economic and social)? What are the potential risks to the environment?	Garth Fletcher
12:00 - 12:15	Meeting Adjourn	

ACKNOWLEDGEMENTS

The Editor wishes to thank the contributions of Peggy Tsang and George Arvanitakis to the organization of this meeting, to Andrew Cogswell and Heinrich Krueger for their excellent skills as rapporteurs, to Nicole Hofs, Jay Parsons, Dan McPhee, and Sarah Cosgrove for editorial assistance in preparation of the Report, and to Wendy Tymchuk and Carlo Biagi for assistance at the meeting venue. Financial contribution from the Canadian Biotechnology Strategy in support of this meeting is gratefully acknowledged.

BACKGROUND

The purpose of this meeting was to lay a foundation for the federal policy and stewardship framework for the assessment of indirect human health and environmental impacts of genetically modified (GM) aquatic organisms, as mandated by the New Substances Notification Regulations of *Canadian Environmental Protection Act 1999 (CEPA 1999).* The *CEPA 1999* provides the federal government with the authority to address pollution problems on land, in water, and through all layers of the atmosphere. The New Substances Notification Regulations (NSNRs) specify the prescribed information to be submitted if a substance (i.e., chemical, polymer, or product of biotechnology) intended for import or manufacture, is not on the Domestic Substances List (DSL). Biotechnology is defined in *CEPA 1999* as "the application of science and engineering in the direct or indirect use of living organisms or part of products of living organisms in their natural or modified forms".

In recent years, there has been an increasing amount of research and development with genetically modified aquatic organisms (particularly GM fish) which may be intended for commercial applications in future. Presently, the authority for the assessment of environmental and indirect human health effects of genetically modified aquatic organisms rests with *CEPA 1999* and the responsibility for carrying out these assessments lies with the New Substances Program of Health Canada and Environment Canada. Drawing upon the knowledge and experience of experts in fisheries biology and genomics, strain enhancement, modeling, environmental effects, and regulatory and policy development, we can strengthen the scientific foundation for the risk assessment of these organisms. The information gathered at this meeting on GM fish and other aquatic GMOs is necessary to ensure that this area is fully considered when a coherent Canadian (federal) government stewardship approach is being formulated. This component on the stewardship for genetically modified aquatic organisms, complements parallel efforts on livestock animals/products, feed, and food derived from animal biotechnology.

PERSPECTIVE OF GENETICALLY MODIFIED FISH USE AND REGULATION IN CHINA

Zuoyan Zhu and Cuizhang Fu

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Abstract

The first successful case of producing transgenic fish in China was in 1985. In the late 1980s, the integration and the expression of the foreign gene have been thoroughly studied in a model system with common carp (*Cyprinus carpio*) as the host fish and a recombinant human growth hormone (hGH) gene as transgene. Compared with non-transgenic peers, hGH-transgenic fish are superior in dietary utilization and growth performance. In view of biosafety and bioethics, an "all-fish" construct CAgcGH, grass carp growth hormone gene fused with common carp β-actin promoter, has been generated and transferred into fertilized eggs of Yellow River carp (*C. carpio*, local strain in Yellow River). Under middle-scale trial, CAgcGH-transgenics show higher growth rate and food conversion efficiency than the control, which is consistent to the laboratory findings. To avoid the potential impact of transgenic fish on the environment, sterile transgenic fish has been successfully produced by triploid manipulation. The "all-fish" transgenic common carp has proven to be safe as daily food, according to a test based on the pathological principles for new medicines issued by the Ministry of Health of China. The "all-fish" transgenic common carp with growth enhancement is now ready for market, but looking for governmental authorization. The framework of committees and legislation that regulate and provide advice on GM organisms is comprehensive in China.

Résumé

Le premier poisson transgénique chinois a été produit en 1985. À la fin des années 1980, l'intégration et l'expression d'un gène étranger avaient été étudiées de manière exhaustive dans un système modèle où la carpe commune (*Cyprinus carpio*) était le poisson-hôte et le transgène était un gène de l'hormone de croissance recombinante humaine (*rhGH*). Les poissons porteurs du transgène *rhGH* sont supérieurs aux poissons non transgéniques sur le plan de l'assimilation et de la croissance. En ce qui a trait à la biosécurité et à la bioéthique, un gène hybride « tout poisson » *CAgcGH*, c.-à-d. le gène de l'hormone de croissance de la carpe de roseau fusionné avec un promoteur de la β-actine de la carpe commune, a été produit et transféré dans des œufs fécondés de carpes du fleuve Jaune (*C. carpio*, souche locale dans le fleuve Jaune). Dans le cadre d'un essai à échelle moyenne, les poissons transgéniques (*CAgcGH*) ont montré des taux de croissance et de conversion alimentaire supérieurs à ceux des témoins, ce qui concorde avec résultats obtenus en laboratoire. Afin d'éviter les répercussions possibles des

poissons transgéniques sur l'environnement, une souche stérile de poissons triploïdes transgéniques a été créée avec succès. Les carpes communes transgéniques (« tout poisson ») se sont révélées être salubres pour la consommation, d'après un test fondé sur les principes pathologiques pour les nouveaux médicaments élaborés par le ministère chinois de la Santé. La carpe commune transgénique à croissance accélérée est maintenant prête pour le marché, mais le gouvernement doit d'abord accorder une autorisation à cet égard. En Chine, le cadre des comités et des lois qui régissent les organismes génétiquement modifiés et fournissent des conseils relatifs à ceux-ci est très complet.

Introduction

With the expansion of the global population and overfishing, advanced aquaculture is needed to meet man's increasing demands for high quality fish protein. Genetically modified (GM) fish (transgenic fish) offer the opportunity to improve both the production and characteristics of conventional fish strains currently exploited in aquaculture. Since Zhu et al. (1985) produced the first batch of fast-growth transgenic fish, many laboratories throughout the world have been successful in generating transgenic fish in a variety of species using different foreign gene constructs. At present, the biotechnology has sufficiently advanced in producing transgenic animals, and fish may be considered the best candidate for the first marketable transgenic animal for human consumption (Zbikowska 2003). The United States Food and Drug Administration (FDA) are looking into the authorization of a fast-growth transgenic salmon (Niiler 2000). Growth enhanced transgenic fish are near the point of application to aquaculture, as such, the governments and public from many countries pay more attention to the safety and nutritional value of the fast-growth transgenic fish.

Comprehensive reports have been recently issued on biosafety of transgenic animals including fish by the Royal Society of Canada (2001), the US Office of Science and Technology Policy (2001), the US National Research Council (2002), the Pew Initiative on Food and Biotechnology (2003), the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (2003). In China, the government, public and scientists also concerned about the bio-safety of transgenic fish. To overcome these concerns, it is important to develop a sound, reliable, and widely accepted method of estimating the potential for harm caused by GM fish escaping into the wild. Recently, the National Natural Science Foundation of China (NSFC) and the Ministry of Science and Technology of China have funded Chinese scientists to assess the biosafety of a fast-growth transgenic common carp including "all-fish" growth hormone gene constructs. In this review, we will introduce advances on transgenic fish studies in China, biosafety considerations of fast-growth GM fish, and Chinese regulations of GM fish.

Transgenic fish studies in China

Integration, expression, and inheritance of transgene

In the late 1980s, the integration and the expression of a foreign gene have been thoroughly studied in a model system with common carp (*Cyprinus carpio*) as the host fish and a recombinant human growth hormone (hGH) gene as transgene (Zhu et al. 1989). The study found that the integration of the foreign gene occurs throughout the course of embryogenesis. The replication of the foreign gene started immediately once introduced into the fertilized eggs. The integration of the foreign gene was supposed to take place at an early stage and last for a long course of time. This resulted in the transgenic mosaicism, i.e., the integrated foreign genes were distributed in different tissues and organs of the transgenic fish. Only those foreign genes integrated into the genome of germlines could be transmitted to the offspring via sexual reproduction. The transcription of the foreign gene could be observed at the late-gastrula stage and radio immunity analysis revealed that different individuals had different levels of foreign gene expression. According to the positional effects related to the expression and function of transgene, the manners of foreign gene integration were divided into three categories: functional integration, silent integration, and toxic integration. Compared with silent integration that does not show any effect and toxic integration that blocks the normal development, only functional integration results in the normal expression of *hGH* (Zhu et al. 1989).

Due to the mosaic integration of the transgene in the founder fish, the frequencies of transgene transmission to F_1 progeny are usually less than that in Mendelian ratios. When "all-fish" GH transgenic carp founders were crossed over non-transgenic controls, the transgenic ratios in the F_1 generation were from 72% to 88%, and it was deduced by genetic analysis that 2-3 chromosomes of each founder were integrated with transgenes (Wang et al. 2001). No matter how the integration of transgene occurs and how many integration sites the transgenic founder posesses, it is necessary to establish a transgenic line with stable germline transmission. Many studies revealed that F_1 transgenics derived from a transgenic founder and non-transgenics were in a heterozygous state, since they gave birth to the next generation with a transgene positive ratio of about 50% (Stuart et al. 1988; Culp et al. 1991; Hew et al. 1992; Lin et al. 1994). Recently, we also found that frequencies of transgene transmission to F_2 progeny from a fast-growth "all-fish" GH F_1 transgenic germline were about 50% in our lab (Zhu et al. unpublished). To mate a pair of siblings of F_2 transgenics from the same parents has shown to be an efficient way to establish a homozygous line of transgenic fish (Culp et al. 1991; Hew et al. 1999). In another study, common carp, transgenic for mouse metallothionein-1 controlled human growth hormone (MThGH) were mated to each other and reproduced generation by generation, which led to the F_4 offspring. It was reported that most transgenes were steadily inherited during four generations' transmission, though a minor proportion of the transgenes were rearranged, deleted and/or inserted with host sequences, and appeared to be highly polymorphic (Zeng et al. 2001). It was also detected that a MThGH-transgene

in the F_4 generation could still initiate transcription properly, and resulted faithful transcripted products (Sun et al. 2000). Therefore, after several generations' transmission, the transgene tends to be stably inherited and functions as an endogenous gene. This phenomenon could be called the "endogenous domestication" of a novel gene.

Growth enhanced transgenic fish

In the late 1970s, Chinese scientists transferred the total DNA of common carp into a tropical species, mud carp (*Cirrhina molitorella*) using the nuclear transplantation method. About 8% of the total DNA transferred mud carp showed improvement on cold-resistance (Zhu and Huang, unpublished data). In the mid 1980s, a study group on transgenic fish lead by Zuoyan Zhu successfully transferred a recombinant human growth gene under the control of mouse metallothionein-1 (MThGH) into the fertilized eggs of goldfish (Zhu et al. 1985) and loach (Zhu et al. 1986), which led to the birth of fast-growth transgenic fish.

For application purpose an "all-fish" genomic construct was constructed including common carp β-actin gene, CA (Liu et al. 1990), and grass carp growth hormone gene, gcGH (Zhu et al. 1992). This "all-fish" GH construct (pCAgcGH) was microinjected into the fertilized eggs of Yellow River carp, a local strain of common carp, and the growth performance was examined at different growth stages (Wang et al. 2001). The study found that the frequency distribution of body weight (BW) of non-transgenic fish (n = 359) was normal, and that of transgenics (n = 324) were not normal at 120-day stage. BW of the largest non-transgenic was 1414 g, and that of the smallest was 264 g. BW of the largest transgenics was 2750 g, and that of the smallest was 84 g. 8.7% of the transgenic individuals have higher BW than the largest non-transgenic individual, and 6.4% of the transgenics weighed more than 2 kg, which is more than a 2-fold increase in BW over non-transgenics. Fast-growth transgenic founders were crossed over the non-transgenics. Results showed that BW frequency distribution of F_1 individuals were normal at 80-day stage, with a mean BW of 417.89 ± 79.72 g. Among all F_1 individuals, 60% were above the average BW of the controls (260.4 ± 22.47 g). The study also found that fast-growth individuals of the P_0 transgenic fish had much thicker muscles on the back and an obvious hunch behind the head (Wang et al. 2001). F_1 generation fast-growth transgenic fish were crossed with non-transgenics resulting in a 2.5-fold increase in body weight of the F_2 generation, over that of controls, at age 200 days (unpublished).

Recently, our lab also successfully produced P_0 GH autotransgenic blunt-snout bream (*Misgurnus mizolepis*) including a construct of blunt-snout bream β-actin gene and its GH cDNA. Preliminary studies have shown that some individuals of P_0 GH autotransgenic blunt-snout bream displayed fast-growth (Li 2003).

Bioenergetic analysis of growth enhanced transgenic fish

Why and how can the growth rate of GH transgenic fish be dramatically improved? Is there any difference in body composition between transgenics and controls? A few studies have been carried out to analyze the bioenergetics of transgenic fish and attempt to answer the above questions. Bioenergetic analysis on F_2 MThGH transgenic common carp, as compared with controls, has been extensively studied (Cui et al. 1996). When fed with fresh tubificid species, the energy budget of both transgenic and control common carp can be expressed by the following equations:

(a) F_2 MThGH-transgenic common carp: $100C = 8.9F+0.63U+49.03R+41.44G$;
(b) Control carp: $100C = 7.37F+1.14U+53.36R+38.13G$;

Where C is the total energy from food, F is the energy lost in faeces, U is the energy lost in nitrogenous exaction, R is the energy channelled to metabolism, and G is the energy channelled to growth.

Compared with the controls, transgenic fish had a significantly higher proportion of food energy channelled to G and a significantly lower proportion of that channelled to R and U. The transgenic fish saved 6.62% of the total energy from food for growth improvement. That phenomenon was named after the "fast-growing and less-eating" effect.

Growth and feed utilization by F_4 MThGH-transgenic common carp fed with diets containing 20%, 30% and 40% protein levels have also been carried out (Fu et al. 1998). The study showed that the transgenics had higher specific growth rates than the controls fed diets at each protein level. Feed intake was significantly higher in the transgenics than in the controls fed a low protein diet (20%), however, feed intake did not significantly differ between transgenics and the controls fed diets with 30% to 40% protein levels. It was thus concluded that at a lower dietary protein level, transgenics achieved higher growth rates, mainly by increasing feed intake; but at a higher dietary protein level, transgenics achieved higher growth rates mainly through higher energy conversion efficiency. The study also showed that the transgenics had significantly higher body contents of dry matter and protein, but lower contents of lipid than the controls fed each diet. The apparent digestibility of amino acids tends to be higher in the transgenics than in the controls, especially in fish fed diets with lower protein levels. While taking a look at the whole-body amino acid pattern in transgenics and controls, Fu et al. (2000) found no differences in 17 amino acids between the transgenics and controls.

Field trials of transgenic fish

Although great advances have been obtained for fast-growth GH transgenic fish in China, no GH transgenic strains have been applied for commercial purposes. To determine

whether transgenic fish have a potential for commercial purposes, it is necessary to perform trials in a situation that is approximate and similar to those of commercial aquaculture operations. Recently, our lab has completed a middle-scale trial of "all-fish" CAgcGH transgenic common carp in Wuhan, a major city in central China, with permission from the Ministry of Agriculture of China (Zhu 2000). New rearing ponds with an area of 30 mu (2 hectares) were built by professional aquaculturists. The banks are high enough to withstand floods and the ponds were enclosed with wire netting that protects transgenics from piscivorous birds and other predators. All of the water-in and water-out channels were designed to prevent escapes of transgenic fish. Strictly managed measures were adopted to assure no escapes of transgenic fish. In addition, each pond was equipped with oxygen supplies and auto-feeding machines, which greatly facilitated aquatic rearing. In the spring of 2000, 2-year-old matured founders of "all-fish" GH transgenic common carp were examined with PCR to detect those transgene carriers whose sexual gonads were transgene positive. Two hundred transgenic individuals that had shown significant growth enhancement were artificially spawned to produce F_1 transgenics. The F_1 transgenics were reared in the ponds with a total area of 25 mu (1.67 ha), and the non-transgenics were reared as a control under the same conditions. From 16 June to 7 September 2000, 50 transgenics and 50 controls were sampled for analysis at 20-day intervals. The results showed that transgenics had growth rates of 80%, 55%, 77%, 60%, and 42%, on average, greater than controls in the serial samplings. On 7 September, when the fish were 142 days old, most of the transgenics had reached market size, while the controls needed another year to reach market size. Further, feeding coefficient (total food weight per unit of gained body weight) of the transgenics was 1.10 and that of controls was 1.35. It could be concluded not only that GH transgenic common carp had faster growth rates but also were more efficient at feed utilization than the controls in the pond culture. It is obvious that by switching to fast-growing transgenic common carp, fish farmers can increase the total yield of fish produced, while greatly reducing the feed costs per kilogram of fish produced. One economic estimate shows that cultivation of transgenic fish would increase economic benefits by 125% over cultivation of non-transgenics. Because China has the largest aquaculture production in the world (FAO 2000), and cultivation of common carp counts for more than 10% of its total aquaculture production, increasing the cultivation of transgenic common carp in China would produce huge economic and social benefits. Wu et al. (2003) present a relatively detailed review on fast-growth GH transgenic common carp.

Biosafety of GM fish

Environmental impacts of fast-growth GM fish

As to the issue of environmental safety, there are two contrary opinions. The major hypothesis held by supporters of transgenic fish is that laboratory induced genetic changes are unlikely to be selectively favored in wild populations under natural selection. Since the wild-type species have endured a long course of natural selection, and accordingly, without sustained large scale releases, transgenic fish would not pose any

threat to the natural ecosystem (Knibb 1997). Others, who hold the opposite opinion, believe that the application of transgenic fish in aquaculture introduces potential interactions of transgenic fish to hybridize or compete with other fish in natural ecosystems, and to negatively impact the natural ecosystems (Kapuscinski and Hallerman 1991; Muir 2004). Here, we focus to discuss the environmental safety of fast-growth GH transgenic fish.

In nature, the ability to compete for available food is a key factor determining survival fitness and invasiveness of a genotype. Fast-growth GH transgenic coho salmon (*Oncorhynchus kisutch*) demonstrated increased feed motivation and an increased ability to compete for food than the non-transgenic controls (Devlin et al. 1999). Fast-growth GH transgenic tilapia (*Oreochromis hornorum*) also showed a significantly higher feeding motivation and competitive feeding success when compared to non-transgenic siblings. Devlin et al. (2004) further found that when transgenic and non-transgenic salmon were cohabitated and competed for different levels of food, transgenic salmon consistently outgrew non-transgenic fish and could affect the growth of non-transgenic cohorts except when food availability was high. When food abundance was low, dominant individuals emerged, which were invariably transgenic. These fish directed strong agonistic and cannibalistic behavior to cohorts and dominated the acquisition of limited food resources. When food availability was low, all groups containing transgenic salmon experienced population crashes or complete extinctions, whereas groups containing only non-transgenic salmon had good (72.0%) survival, and their population biomass continued to increase. However, Guillén et al. (1999) reported that the wild type tilapia showed a significantly higher feed motivation and an increased ability to compete for food than transgenic tilapia. So, the different fast-growth GH transgenic fish may display the different ability to compete for available food compared with the wild type.

Fast-growth GH transgenic channel catfish (*Ictalurus punctatus*) displayed poorer anti-predatory behavior than non-transgenic controls (Dunham et al. 1999). The same effect was also found in a behavior study of fast-growth GH transgenic Atlantic salmon (*Salmo salar*) (Abrahams and Sutterlin 1999). A recent study further showed that vertical position generally did not differ between fast-growth GH transgenic and normal coho salmon fry, but at larger size (>4 g) transgenic fish remained closer to the surface than the controls (Sundström et al. 2003). In nature, where predators may attack from above (birds) or below (fish), this kind of behavior may translate into higher risk of predation, which could increase mortality and lower the fitness of transgenic fish, unless their increased growth rate can compensate for the increased risk-taking (Sundström et al. 2003). Based on these results, fast-growth GH transgenic fish display poorer anti-predatory behaviour, and do suggest that they will suffer higher mortality rates under natural conditions.

At the same body size, individuals with a high intrinsic growth rate will perform a variety of functions less efficiently than slow-growing animals (Morgan et al. 2000). For instance, at the same length, fast-growing fish swim less efficiently than slow-growing

fish (Kolok and Oris 1995; Gregory and Wood, 1998 1999). Fast-growth GH transgenic juvenile coho salmon grow more than twice as fast by length as the controls, but have critical swimming speeds half those of the controls of the same length (Farrell et al. 1997). A study of Lee et al. (2003) also showed that fast-growth GH transgenic adult transgenic coho salmon displayed significantly lower critical swimming speeds than the adult controls. In addition, the study further showed that transgenic fish are less economical swimmers at all swimming speeds. Stevens et al. (1998), however, did not find any significant differences in swimming ability between transgenic Atlantic salmon and controls. Such differences may be attributed in part to the degree of GH enhancement that exists between different transgenic strains and species. Further tests with other fast-growth GH transgenic fish may serve to ultimately clarify this. Differences in swimming ability have important implications for risk assessments of transgenic fish, since this performance characteristic influences many life-history characteristics including catching prey, escaping predators, and migrating up spawning habitats. Impairment of any of these capabilities in fast-growth GH transgenic fish could seriously affect their fitness relative to wild counterparts (Lee et al. 2003).

Somatic development may compromise rapid growth because most cells lose the ability to divide and contribute further towards growth once they differentiate and take up their mature function (Starck and Ricklefs 1998). In fishes, a fast growing strain of pumpkinseed sunfish (*Lepomis gibbosus*) had a delayed onset of mineralization in their cranial bones relative to a slow growing strain (Arendt and Wilson 2000). Further study found a negative correlation between scale strength (in terms of ability to resist being pierced) and growth rate that was consistent both within and among populations using pumpkinseed from six populations known to differ in their intrinsic growth rates (Arendt et al. 2001). This trade-off between growth rate and scale strength may have fitness consequences in terms of likelihood of surviving predation attempts or swimming efficiency. This may in part explain the trade-offs between growth rate and critical swimming speed recently found in fast-growth GH transgenic coho salmon (Farrell et al. 1997; Lee et al. 2003). Arendt et al. (2001) suggested that if the trade-offs between growth rate and scale strength holds for all skeletal elements in fish, feeding efficiency may be severely compromised in molluscivores. Adult common carp mainly feed on the mollusc. If, due to rapid growth, their teeth are too low density to crush prey, then we may expect that fast-growth GH transgenic common carp will be confined to smaller snails relative to wild counterparts if released to the natural environment. It further suggests that fast-growth GH transgenic common carp may have narrow range of food resources in contrast with its wild counterparts.

A trade-off is expected between growth rate and immunological competence, because of the substantial nutritional and energetic costs associated with immunological stress and the maintenance of an efficient immune system (Lochmiller and Deerenberg 2000). Information on disease resistance of fast-growth *GH*-transgenic fish is limited. Fast-growth “all-fish” GH-transgenic common carp promoted thymus development and thymocyte proliferation, and retarded thymus degeneration (Guo et al. 2003). Resistance

to the bacterial pathogen was not affected in fast-growth *GH*-transgenic coho salmon relative to their non-transgenic counterparts when they were infected at the fry stage, but was lower in transgenic fish when infected near smolting (Jhingan et al. 2003). Reduced disease resistance in transgenic fishes at any developmental stage could have important implications on the impact of escapees on wild populations (Devlin and Donaldson 1992). Along with reducing disease resistance, if the transgenics reduce the probability of survival, then the transgenics would probably not persist in nature.

Age at sexual maturity is a critical fitness character for animals. It determines the number of possible generations over time. Fast-growth GH transgenic salmon have been reported to show a lower age of sexual maturity (Devlin et al. 2004). However, we recently found that sexual maturity in non-transgenic carp male individuals occurred at age 6 months (body mass = 1079 ± 46 g) and sexual maturity in the fast-growth "all-fish" GH transgenic carp male individuals still did not occur at age 7 months (body mass = 3143 ± 405 g) (unpublished). The results indicated that fast-growth "all-fish" GH transgenic carp did not lower the age of sexual maturity.

In a study conducted with fast-growth "all-fish" GH transgenic common carp, the gonadosomatic index (ovary weight/body weight), fertilization rate (number of fertilized eggs/number of eggs) and hatchability (number of hatched fry/ number of fertilized eggs) were compared with wild-type non-transgenics. Results showed that the quality of eggs of transgenics was similar to that of the non-transgenics, while the gonadosomatic index of the transgenics was significantly lower than that of the nontransgenics. For both transgenics and non-transgenics, while the fertilization rate was more than 80% and hatchability was more than 60%, there were no significant differences in these parameters between the transgenics and non-transgenics (Wang et al. 2001).

The above-mentioned findings may indicate that even if fast-growth GH transgenic fish were released in nature by accident, transgenic fish would likely have little or no impact on the natural ecosystem. However, even if not well adapted for survival in the wild, transgenic fish may have detrimental impacts on the genetic structure of wild populations by allowing the introgression of exotic genes into natural gene pools. It has been hypothesized that if transgenic males have increased mating success but their transgenic offspring have decreased viability, releasing transgenics to natural populations may cause the eventual local extinction of both populations. The explanation is that the male mating advantage would increase the frequency of the transgene in the population, and the viability disadvantage suffered by all of the offspring carrying the transgene would reduce the population size (Muir and Howard 1999, 2001, 2002; Howard et al. 2004). Thus, until extensive risk evaluations have been made for transgenic fish, they should be reared only in circumscribed ponds and not applied to aquaculture.

In summary, the distinct phenotypic characteristics of GH transgenic species suggest that evaluation for aquaculture and for risk assessments requires examination of strains on a

case-by-case basis. In China, complete risk assessments for "all-fish" GH transgenic carp will be conducted in the following years.

In addition, there are two ways to deal with the possible risk of transgenic fish escaping into natural ecosystems by genetic methods. The first and most direct way is to stock transgenic fish in relatively isolated and strictly managed ponds, which could prevent the escape of transgenic fish. It is obvious that this solution would be expensive in terms of both labor and material resources. This would, to a considerable degree, counteract the benefits of cultivating transgenic fish. The second solution, and also the ultimate one, is to produce sterile transgenic fish for which several approaches have been described (Devlin and Donaldson 1992; Thorgaard et al. 1992; Rahman et al. 1998; Zhu and Sun 2000; Hew and Fletcher 2001; Melamed et al. 2002). A discussion of this approach for using sterile transgenic fish follows.

Producing sterile transgenic fish

Polyploidy manipulation techniques are easily applied to fish, which offers fish biologists an approach for producing various useful reproductive characteristics for commercial aquaculture. In many fish species, triploid males and females generally fail to produce mature gonads and turn out to be sterile. To reduce the environmental impact of transgenic tilapia, triploidy had been induced in two lines of transgenic fish by heat-shock (Razak et al. 1999). Observations of growth revealed that, though transgenic triploids showed a little decrease in growth performance compared with transgenic diploids, they grew faster than non-transgenic diploids and non-transgenic triploid. The transgenic triploid males produced some spermatozoa in some cases. The ovaries of all triploids, however, were devoid of oocytes and were shown to be completely nonfunctional. The authors proposed that triploid transgenic female tilapias were perfect for aquaculture, since they showed a higher growth rate than the normal wild-type tilapias, without the risk of any genetic impact on the local gene pool.

In China, a study of interspecific hybridization between red crucian carp (*Carassius auratus* red var.) and common carp found that the F_3-F_8 generations of the hybrids were allotetraploids and viable to produce tetraploid offspring (Liu et al. 2001). An "All-fish" GH construct has been transferred into the fertilized eggs of the allotetraploids, and the transgenic tetraploids showed significantly superior growth performance over non-transgenic tetraploids. Transgenic tetraploids could produce spermatozoa when they were 240 days old (Zeng et al. 2000). Transgenic triploids were then successfully produced by crossing transgenic diploid common carp over tetraploids (Zhu 2000). Transgenic triploids were found to be sterile and had higher growth rates than non-transgenics. Since transgenic tetraploids are able to produce successive generations of offspring and still maintain the tetraploidy, the technology is available to produce transgenic triploids for aquaculture with no environmental impacts. This method is also considered to be more convenient and more readily applicable than the method of heat-shock or pressure-shock induced triploidisation.

It is obvious that the triploid strategy is not suitable for all species of transgenic fish, and more feasible techniques should be developed to ensure the environmental safety of transgenic fish. It is well known that fish gonadotropin-releasing hormones (GnRHs) are decapeptides that play critical roles in fish gonadal development and in regulation of the reproductive cycle (Alestrom et al. 1992; Uzbekova et al. 2002). Repressing the expression of GnRHs is likely to lead to sterility of the fishes. The most promising method for repressing genes at present appears to be through antisense technology, which has only achieved commercial success in transgenic plants (van der Meer et al. 1992; Schmulling et al. 1993). Introduction of short DNA or RNA sequences corresponding to part of the coding sequence can block the expression of a particular gene, either by binding the double stranded DNA to form a triplex, to block transcription, or by binding the mRNA, to block its processing and transportation. By introducing constructs containing salmon GnRH promoter fused to GnRH antisense cDNA into rainbow trout, Uzbekova et al. (2000) found the expression of antisense GnRH RNA in the brain and a decrease in the production of endogenous GnRH mRNA in the brain and pituitaries. Unexpectedly, the levels of the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), were not affected in the antisense GnRH transgenics, and the fish reached maturation at the same time as nontransgenic individuals. On the other hand, when histone H3 promoter was used to drive antisense GnRH cDNA, some transgenic rainbow trout were sterile, and their fertility could be restored by hormonal treatment (Uzbekova et al. 2001). In our lab, common carp derived GnRH cDNA was isolated, and a construct with common carp β-actin promoter and antisense GnRH cDNA were subsequently generated. Introducing this antisense GnRH construct into fast-growing transgenic common carp may lead to a sterile strain, which would finally solve the problems involved in environmental safety of transgenic fish. Furthermore, the fertility of antisense GnRH-transgenics with desirable performance could be restored by exogenous hormone administration, resulting in a fertility reversible strain of transgenic fish that could serve as brood stock for aquaculturists (Li 2004).

Food safety of transgenic fish

At present, the widely accepted principle for evaluating the safety of foods produced by modern biotechnology is the “substantial equivalence principle”, which was proposed by the European Organization for Economic Cooperation and Development in 1993 (OECD 1993). In 1995, a World Health Organization (WHO) consulting group convened to provide practical guidance for the safety evaluation of plants derived from modern biotechnology, and they used the substantial equivalence principle in this guidance (WHO 1995). In 1996, the WHO and the Food and Agriculture Orgnaization (FAO) convened together to provide practical and concrete recommendations for international guidelines for the safety assessment of foods derived from biotechnology, and suggested that the substantial equivalence principle be used as a general guidance for the safety evaluation of all GMOs (FAO/ WHO 1996). There was no evidence that “all-fish” GH-transgenic common carp produced any new proteins or other new biological products.

Strictly speaking, they only produce higher levels of piscine growth hormone, when compared with wild-type non-transgenic fish. Therefore, according to the principle of substantial equivalence, "all-fish" GH-transgenic common carp should have been classified under the safest level, i.e., level-I (Zhu and Zeng 2000). Nevertheless, their food safety was evaluated using three groups of mice that were each fed with "all-fish" GH-transgenic common carp, non-transgenic common carp, and physiological saline for six weeks. The experiments strictly followed the pathological rules for testing new medicines issued by the Ministry of Health, People's Republic of China (P. R. China). The results indicated that the test mice did not show any significant differences in terms of growth performance, biochemical analysis of blood, histochemical assay of 12 organs, reproductive ability, etc., when compared with two groups of control mice (Zhang et al. 2000). It should be concluded that "all-fish" GH-transgenic common carp is safe enough to be eaten daily. In another safety evaluation of fast-growth GH-transgenic tilapia, researchers employed long-tailed macaques (*Macaca fascicularis*) as test animals, and found that the tilapia growth hormone had no biological activity when administered to non-human primates. Twenty-two human individuals volunteered to eat transgenic tilapia for one week, and no effects were detected in the volunteers' health (Guillén et al. 1999). The most recent GM fish that may have been developed are transgenic only with respect to sequences obtained from fish of the same species (Nam et al. 2001). That is, they are autotransgenics; similar works by our labotary in blunt-snout bream, and Maclean and colleagues in tilapia, are currently at an advanced stage. There seems no reason to doubt that such fish may be safe to eat and could indeed be an improvement on currently available farmed fish, which are regularly exposed to antibiotics, injected vaccines with adjuvants, and food additives, such as carotene in feed pellets (Maclean 2003). Taste testing has been reported of GM tilapia in Cuba (Guillen et al. 1999) and GM trout in Canada (Entis 1998). It could be concluded that "all-fish" transgenic fish are not only safe to eat but also very delicious.

Regulations of GM fish

The framework of committees and legislation that regulate and provide advice on GM organisms is comprehensive in P. R. China. All GM organisms developed in China, regardless of their intended use, must be assessed by the committees and legislation. These are outlined briefly here.

On December 24, 1993, the first regulation, "Safety Administration Regulation on Genetic Engineering" was issued by State Science and Technology Commission of China. This regulation aimed at promoting research and development of biotechnology in China, tightening safety control of genetic engineering work, guaranteeing public health of common citizens and genetic engineering workers, preventing environmental pollution, and maintaining ecological balance. It stipulated concretely on the management of technologies utilizing carrier systems to reorganize DNA and importing the DNA from other sources into the organisms in physical and chemical ways.

On July 10, 1996, the second regulation "Safety Administration Implementation Regulation on Agricultural Biological Genetic Engineering" was issued by the Ministry of Agriculture, P. R. China. This regulation makes classification of safety of genetic engineering carriers, and provides relevant management measures. Especially, it designates the procedures and rules of the registration and safety assessment of agricultural biological genetic engineering. The regulation is carried out effectively in the management of agricultural biological genetic engineering across the country. The above-mentioned two regulations are available at http://www.biosafety.gov.cn.

On May 23, 2001, the Chinese State Council promulgated "the Regulation of Biosafety Management on Agricultural Transgenic Organisms". This regulation stipulated what the procedure is for obtaining permission to undertake research and trial of GM organisms.

On March 20, 2002, "Labeling Administration Regulation on Agricultural Transgenic Organisms" was issued by the Ministry of Agriculture, P. R. China. This regulation demands that all listed transgenic biological products should be labeled in order to protect consumers' rights to information and choice in China.

On April 8, 2002, "Health Administration Regulation on Transgenic Food" was issued by the Ministry of Health, P. R. China. This regulation regulates, licenses and monitors all GM food from the human safety point of view.

On July 8, 2002, "the National Transgenic Biosafety Committee of China" was founded. The committee is responsible for the biosafety assessment of GM organisms, biotechnology consultation and supervision.

All above-listed regulations are used to regulate, license and monitor use of GM fish in China. At some point in the future, should a scientist or company wish to market a GM fish for food with the achievements of sterility-reversible transgenic fish and the environmental release testing, they must apply to the National Transgenic Biosafety Committee of China.

Conclusion

In China, GM fish are being developed primarily to produce desirable alterations to growth rates or feed conversion efficiency. Up to the present, no transgenic animals, and undoubtedly, no transgenic fish have been commercially produced or approved for human consumption. The Chinese government and scientists are very cautious in assessing the biosafety of GM fish. Though some above-mentioned studies have shown that fast-growth "all-fish" GH transgenic common carp is safe for human consumption and have no negative impact on the environment, further studies will be conducted to comprehensively assess the environmental impacts and food safety of the fast-growth GM carp. Those involved in the technology, whether in the development of legislation or

in the application of the scientific developments, should engage in an open and frank debate with the public to recognize and address public concerns of the about these issues.

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Participant Discussions

Muir: Elaborate more on the RNA antisense research. I couldn't understand what the construct was, what it was knocking out and what it did.

Zhu: The construct, pCAgcGHc antisense 6754 bp, was cloned from a carp promoter and terminator. There are two types of GnRH, chicken gene and salmon type. The construct blocks GnH production and inhibits gonadal growth.

Muir: You would expect then that the antisense would knock out reproduction in males and females?

Zhu: Only in males. We saw 32% of males fertile in general for the control. Of the fish squeezed in the founders, only 33-34% of fish produced sperm. In females, only 50% have eggs.

Muir: Is the antisense leaky?

Zhu: Yes, somewhat.

Unknown: Is this data for founders, not the following generations?

Zhu: This is for the founder. Why they are not completely sterile in founders, is not known, but this may be due to mosaic integration.

Muir: Is the construct antisense for the entire DNA sequence or just some region of it essential to function?

Zhu: We checked the hormone sequence and compared the conserved regions of cDNAs of different species of fish. It is a short sequence, with not many base pairs.

Fletcher: What's the next step if you cannot restore reproduction of founders?

Zhu: This study is just to prove we could do it. Future work will reveal whether this research is applicable in subsequent generations.

Bughio: I have a question regarding GMOs in China. Are there regulations for containment and disposal of transgenic fish?

Zhu: Yes, there are very tight regulations; otherwise all fish would be transgenic fish. The Chinese government is not going to approve biotech fish for human consumption in the near future. They are waiting for other governments to take the lead on this.

Tsang: Does the Chinese government require full containment of transgenic fish?

Zhu: Yes.

Bughio: If there are any fish that die, how do you dispose of them?

Zhu: We kill all the fish and we throw them away. We have no special place to collect fish.

Bughio: Is there a special facility for disposal?

Zhu: No, we just bury them after they are killed.

Devlin: Do you see any behavioral changes in the transgenic fish in terms of feeding?

Zhu: They seem to move more slowly and the workers can easily identify them because the transgenics seem to come to the feeding area more often.

Devlin: Do the tetraploid parents ever produce haploid gametes?

Zhu: Diploid only. Especially in the males with a very small amount of sperm produced.

TRANSGENIC MODIFICATION AND OTHER BIOTECHNOLOGY APPROACHES TO STRAIN ENHANCEMENT FOR AQUACULTURE

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Abstract

There are many ways to genetically modify fish including inbreeding, gynogenesis, androgenesis, selection, intraspecific crossbreeding, interspecific hybridization, polyploidy, sex reversal and breeding, nuclear transplantation, and transgenesis. Domestication and strain effects are keys that affect the success of all of these genetic enhancement programs. Various combinations of these programs can improve important aquaculture traits such as growth, feed conversion, survival, disease resistance, tolerance of poor water quality, harvestability, carcass quality and quantity, coloration, and reproduction. Transgenesis appears to have the potential to enhance growth and disease resistance to a greater extent than other genetic enhancement programs; however, there appears to be a highly variable response by species with species having relatively slow growth benefiting the most. Maximum attainable size was quadrupled in mud loach (*Misgurnus mesolepis*) by introducing growth hormone genes. However, little data are available in experimental units that simulate commercial aquaculture conditions. Initial data indicates that the ultimate aquaculture genotypes will likely result from utilizing a variety of genetic enhancement programs. Fitness traits examined to date in aquaculture species of transgenic fish indicate a lowered fitness of these fish, although more data are needed. Triploidy is a potential method to sterilize production animals of transgenic fish of some species, but still requires fertile brood fish and the polyploidy can reduce the extent of the transgenic enhancement. Improved genetic sterilization needs to be developed.

Résumé

Il existe de nombreuses façons de modifier génétiquement le poisson, notamment l'autofécondation, la gynogénèse, l'androgenèse, la sélection, le croisement intraspécifique, l'hybridation interspécifique, la polyploïdie, l'inversion du sexe et la reproduction, la transplantation nucléaire et la transgénèse. Les effets de la domestication et les effets liés aux souches sont les principaux éléments qui ont une incidence sur la réussite de tous ces programmes d'amélioration génétique. Diverses combinaisons de ces programmes peuvent améliorer des caractéristiques importantes aux fins d'aquaculture, comme la croissance, la conversion alimentaire, la survie, la résistance aux maladies, la tolérance aux eaux de mauvaise qualité, la possibilité de récolte, la qualité des carcasses et leur volume, la couleur et la reproduction. La transgénèse semble être la meilleure

modification génétique pour améliorer la croissance et la résistance aux maladies, mais elle semble donner des résultats très différents d'une espèce à l'autre, les espèces à la croissance relativement lente bénéficiant le plus d'une telle modification. La taille maximale possible de la loche (*Misgurnus mizolepis*) à quadrupler à la suite de l'introduction de gènes de l'hormone de croissance. Il existe cependant peu de données d'études menées en milieu expérimental où les conditions d'aquaculture commerciale sont simulées. Les données initiales indiquent que les meilleurs génotypes aux fins d'aquaculture seront probablement produits en utilisant toute une gamme de programmes d'amélioration génétique. Les caractéristiques de valeur adaptative examinées jusqu'à maintenant chez les poissons transgéniques d'espèces aquacoles montrent que la valeur adaptative de ces poissons est inférieure à celles des poissons non transgéniques, mais d'autres données sont toutefois nécessaires. La triploïdie est une méthode qui pourrait être utilisée pour stériliser les poissons transgéniques de certaines espèces, mais elle nécessite tout de même des reproducteurs fertiles et la polyploïdie peut réduire l'ampleur des améliorations possibles grâce à la transgénèse. Une meilleure méthode de stérilisation génétique doit être élaborée.

Performance of transgenic fish

Growth

The greatest amount of research on transgenic fish and shellfish has focused on transfer of growth hormone (GH) genes. Due to the lack of available piscine gene sequences, transgenic fish research in the mid 1980s employed existing mammalian GH gene constructs, and growth enhancement was reported for some fish species (Zhu et al. 1986; Enikolopov et al. 1989; Zhu 1992; Gross et al. 1992; Lu et al. 1992; Wu et al. 1994). Mammalian gene constructs (mMT/rGH) failed to effect growth of salmonids (Guyomard et al. 1989a,b; Penman et al. 1991), despite the fact that salmonids are very responsive to growth stimulation by exogenously administered mammalian GH protein (Maclean and Donaldson 1993). However, mammalian GH constructs enhanced growth of other fish species as discussed below.

Then, gene constructs containing fish GH sequences driven by non-piscine promoters elicited growth enhancement in transgenic carp, catfish, zebrafish, and tilapia (Zhang et al. 1990; Dunham et al. 1992; Chen et al. 1993; Zhao et al. 1993; Martinez et al. 1996). Several species including loach, common carp, crucian carp, Atlantic salmon, channel catfish, tilapia, medaka, and northern pike containing either human, bovine, or salmonid growth hormone genes were reported to grow 10-80% faster than non-transgenic fish. Transgenic individuals of some families of carp and catfish grew 20-150% faster than their non-transgenic full-siblings, but in some families no differences existed. This variable response by families is a universal observation in transgenic fish. Transgenic common carp and silver crucian carp possessing MThGHg grew 11% and 78% faster than non-transgenic controls, respectively (Chen et al. 1990). The different response of

the two species can be explained by variable expression of individuals, differing insertion sites and therefore differing regulation and expression, or small sample sizes. Traditional selective breeding, sex reversal and breeding and polyploidy also have the potential to double growth rate, but this may take anywhere from 1-10 generations depending upon the genetic enhancement program and the species.

For example, intraspecific crossbreeding often increases growth rate of aquacultured species, but as expected, heterosis is obtained in anywhere from 5-80% of crossbreeding evaluations depending upon species and combining abilities of strains and lines. Crossbred channel catfish sometimes grow 10-30% faster than the largest parental strain (Dunham and Smitherman 1983; Dunham 1996). The best common carp crossbreeds expressed 10-50% increase in growth (Moav et al. 1964; Moav and Wohlfarth 1974; Nagy et al. 1984; Wohlfarth 1993; Bakos and Gorda 1995; Hulata 1995; Gupta et al. 2001); however, those exhibiting heterosis are the basis for carp industries in Israel, Vietnam, China, and Hungary. The silver barb (*Puntius gonionotus*) crossbreed growth was up to 35% higher than that of the pure strains (Gupta et al. 2001).

Selection has been successful in increasing growth rate in a number of species including channel catfish (*Ictalurus punctatus*), several salmonids (*Oncorhyncus sp*.), common carp (*Cyprinus carpio*), tilapias (*Oreochromis sp.*), rohu (*Labeo rohita),* American oyster, (*Crassostrea virginica*), shrimp, and clams (Dunham et al. 2001). The estimated gains vary from 4.4-20% per generation, which is much higher than that usually obtained in farm animals. The best select line grew twice as fast as average strains of channel catfish (Burch 1986). Selection is a consistent technology to improve body weight of aquacultured fish, and usually, but does not always improve body weight (Dunham 1996). In some cases growth has been doubled after several generations of selection. In some species, such as common carp and Nile tilapia, the success of selection is inconsistent. This is not unexpected as genetic background and quantities of additive genetic variation can vary from one population to another. Depending upon the quantity of additive genetic variation and the influences of other contributors of total phenotypic variation, different selection programs, family or mass selection could yield different results. In summary a genetic gain of 10-15 % per generation is possible and growth rate can be doubled in 6 generations.

Polyploidy has been well studied in fish and shellfish. Very rarely does the induction of triploidy enhance growth. However, in species that are sometimes harvested at an age and size when sexual maturity normally occurs in diploids, such as salmonids, oysters and, recently, common carp, the triploid genotype can be superior in growth, flesh quality and carcass yeild.

Sex reversal and breeding to produce monosex populations and obtain the advantages of sexually dimorphic growth has resulted in faster growing Nile tilapia, channel catfish, salmonids, common carp, and silver barb. Results from on-station trials indicate that all-male Nile tilapia have considerable benefits under culture, significantly increasing yields

by up to 58% compared to mixed sex tilapia of the same strain (Mair et al. 1995). Yields of genetically male tilapia, GMT, are also consistently greater than those for sex reversed male tilapia. All-female common carp seed was released to commercial farms and resulted in 10-15% yield improvement over existing commercial stocks (Cherfas et al. 1996).

In comparison to the earliest experiments with transgenic fish and the results of other genetic enhancement programs, later experiments with GH transgenic fish demonstrated that growth can be accelerated through transgenesis from 10% up to an incredible 35-fold in some conditions (Devlin et al. 1994, 1995a,b; Nam et al. 2001; Kim et al. 2002). Du et al. (1992) used an all-fish GH gene construct to make transgenic Atlantic salmon. They reported a 2 to 6-fold increase of the transgenic fish growth rate. Similar results have been obtained for transgenic *Oreochromis niloticus* possessing one copy of an eel (ocean) pout promoter-chinook salmon (*Oncorhyncus tshawytscha*) growth hormone fusion that grew 2.5 to 4-fold faster and converted feed 20% better than non-transgenic siblings (Rahman et al. 1998, 2001; Rahman and Maclean 1999). However, F1 Nile tilapia transgenic for a construct consisting of a sockeye salmon metallothionein promoter spliced to a sockeye salmon growth hormone gene exhibited no growth enhancement (Rahman et al. 1998), although salmon transgenic for this construct show greatly enhanced growth. Insertions of other GH constructs into tilapia have also yielded positive results, but not as dramatic as those with the salmon GH constructs. Possible explanations for the difference in results are the type of construct and the type of tilapia studied were different as well as standard hypotheses such as position effects of the insert in the genome. Introduction of a cytomegalovirus (CMV)/tilapia GH construct into a hybrid *Oreochromis hornorum* resulted in 60-80% growth acceleration (Martinez et al. 1996; Estrada et al. 1999) depending on the culture conditions.

GH gene constructs utilizing entirely piscine gene sequences (using either an ocean pout antifreeze promoter driving a chinook salmon GH cDNA, or a sockeye salmon metallothionein promoter driving the full-length sockeye GH1 gene) gave the dramatic growth enhancement. When introduced into salmonids, these gene constructs elevated circulating GH levels by as much as 40-fold (Devlin et al. 1994; Devlin 1997), resulting in a 5 to 30-fold increase in weight after one year of growth (Du et al. 1992; Devlin et al. 1994, 1995a,b). These gene constructs allowed precocious development of physiological capabilities necessary for marine survival (smoltification). The extraordinary accelerated growth was obtained in number of salmonid species. Ocean pout AFP/chinook salmon growth hormone gene construct accelerated growth in coho salmon (*Oncorhynchus Kisutch*) by 10 to 30-fold (Devlin et al. 1995a). Results varied among species and families and might be related to different gene constructs, coding regions, chromosome positions and copy numbers. Insertion of opAFP-chinook salmon GH1cDNA increased growth 3.2-fold in rainbow trout, whereas the sockeye MTB sockeye GH1 accelerated growth 10-fold (Devlin et al. 1995a; Devlin 1997). The opAFP-chinook salmon GH1cDNA construct improved growth 10-fold in cutthroat trout, *Oncorhynchus clarki*, and 6.2-fold in chinook salmon, O. *tshawytscha* (Devlin et al. 1995a). Growth at 5

months was better than growth at 15 months again illustrating that on a relative basis, genetic improvement for growth is usually more impressive at younger ages than older ages. Condition factor (K), which indicates the robustness of the fish, was lower for transgenics because length changed more rapidly than weight. Some families that had 30-fold increased growth exhibited acromegaly of the jaw, skull and opercular area and by 15 months, the growth of these fish slowed and they died. As was seen with transgenic common carp and channel catfish, the effect of GH gene insertion was variable among families, and multiple insertion sites and multiple copies of the gene were observed.

Sockeye MT-B-sockeyeGHcDNA1 introduced into coho salmon increased growth 11 to 37-fold, and increased GH expression by 40-fold in cold temperatures when GH expression is normally low (Devlin et al. 2001). Results with Atlantic salmon are not quite as impressive as with coho salmon. Transgenic Atlantic salmon containing the ocean pout antifreeze promoter-chinook salmon growth hormone (GHcDNA1) gene construct had a 3 to 6-fold accelerated growth rate compared to non-transgenic salmon (Du et al. 1992; Cook et al. 2000a). Insertion of sockeye MT-B-sockeyeGHcDNA1 (Devlin 1997) produced a similar result, 5-fold growth enhancement.

Prior to first feeding, the transgenic progeny were found to be 21.2% heavier and 11.9% longer than their non-transgenic siblings, suggesting that the expression of GH in early development can influence the rate or efficiency of conversion of yolk energy reserves (Devlin et al. 1995a,b).

Magnification effects can explain some of the growth differences between transgenic and control salmon. However, specific growth rates of the transgenic coho were approximately 2.7-fold higher than older nontransgenic animals of similar size, and 1.7-fold higher than their nontransgenic siblings (Devlin et al. 2000) indicating that the transgenic salmon are growing at a faster rate at numerous sizes and life stages. GH levels were increased dramatically (19.3 to 32.1-fold) relative to size control salmon, but IGF-I levels were only modestly affected, being slightly enhanced in one experiment and slightly reduced in another.

Transgenic mud loach, *M. mizolepis*, that were transgenic for mud loach growth hormone (mlGH) gene fused to the mud loach beta-actin promoter had dramatically accelerated growth, up to a maximum of 35-fold faster than their non-transgeni**c** siblings (Nam et al. 2001). Many fast-growing transgenic individuals exhibited extraordinary gigantism. The body weight and total length (maximum of 413 g and 41.5 cm) were greater than even those of 12-year-old non-transgenic brood stock (maximum size of 89 g and 28 cm). Enhanced growth was variable among transgenic lines as has been the case in all earlier transgenic fish studies. The time required to attain marketable size (10 g) for transgenic lines was only 30-50 days after fertilization, compared to at least 6 months for controls. A maximum of 1.9-fold improved feed-conversion efficiency was also observed.

Devlin et al. (2001) concluded that growth-hormone constructs that work well in a relatively slow growing cold water species such as salmon may not work as well in a relatively rapid growing species such as tilapia, and that the phenotypic and genetic character of the starting species or strain, as well as the strength of the gene construct, need to be considered when attempting to improve aquacultured fish. With this in mind, the dramatically enhanced growth exhibited by the transgenic salmon and loach is not impressive when compared to the potential growth of non-transgenic channel catfish and common carp under good environmental conditions. Perhaps the less dramatic improvement of GH transgenic catfish and carp is due to them being closer to some type of biological maximum for freshwater fish growth rate. However, the explanation must be more complicated than this as there are several freshwater species that have much greater maximum growth rates than channel catfish or common carp.

Cold tolerance

Early research on transgenic fish also involved the transfer of the antifreeze protein gene of the winter flounder, *Pleuronectues americanus* (Fletcher et al. 1988). The primary purpose of this research was to produce salmon that could be farmed under arctic conditions, but expression levels obtained have been inadequate for increasing cold tolerance in salmon (Hew et al. 1999). However, preliminary results with goldfish show some promise for increasing survival within the normal cold temperature range. One copy of a major liver-type antifreeze protein gene was integrated into a single salmon genome. The antifreeze protein mRNA was only expressed in the liver and showed seasonal variation in F3 transgenic progeny and all individuals expressed similar levels of the antifreeze protein precursor protein in the sera. The sera of the transgenic offspring showed characteristic hexagonal ice crystal pattern indicating the presence of antifreeze activity, whereas controls exhibited the expected round ice crystal pattern indicating no antifreeze activity. The antifreeze protein precursor level was highest in the month of November and lowest in May. The ability of these transgenic salmon to survive lethal temperatures was not improved. The antifreeze protein level was only 10-20% of that expressed by winter flounder. Winter flounder have multiple copies of the antifreeze protein gene, which may be part of their mechanism to produce antifreeze protein at levels that provide protection of the blood. Initial thrusts at improving cold tolerance via gene transfer have been minimally successful and expression will have to be increased to obtain the desired biological effect of freeze protection.

Neither has selective breeding been very successful for improving cold tolerance. Interspecific hybrids may have increased environmental tolerances when one parental species has a wide tolerance such as euryhaline species, a specific tolerance such as cold tolerance, or because of increased heterozygosity (Nelson and Hedgecock 1980; Nevo et al. 1986). Interspecific backcrossing has been used successfully to transfer improved cold tolerance from one species to another, but the net overall improvement compared to best parent species is minimal in the interspecific backcross (Behrends and Smitherman 1984).

Disease resistance

Momentum is being gained in transgenic enhancement of disease resistance. Expression of viral coat protein genes (Anderson et al. 1996) or antisense of viral early genes may improve virus resistance. Bacterial disease resistance may be easier to genetically engineer than for diseases caused by other classifications of pathogenic organisms.

Bacterial disease resistance may be improved up to 3 to 4-fold through gene transfer. Insertion of lytic peptide cecropin B construct enhanced resistance to bacterial diseases 2 to 4-fold in channel catfish (Dunham et al. 2002d). There was no pleiotropic effect on growth. Transgenic and non-transgenic full-siblings, containing the cecropin B construct were challenged in tanks with *Edwardsiella ictaluri*. Both genotypes experienced mortality, but the survival of the transgenic individuals was twice that of the controls. Transgenic channel catfish containing the preprocecropin B construct and their full-sibling controls experienced a natural epizootic of columnaris*, Flavobacterium columnare*. No cecropin-transgenic fish were among the mortalities, and only control fish died.

Similar results were obtained for cecropin transgenic medaka (*Oryzias latipes*) (Sarmasik et al. 2002). F2 transgenic medaka from different families and controls were challenged with *Psuedomonas fluorescens* and *Vibrio anguillarum* killing about 40% of the control fish by both pathogens, but only 0-10% of the F2 transgenic fish were killed by *P. fluorescens* and about 10-30% killed by *V. anguillarum*. When challenged with *P. fluorescens*, zero mortality was found in one transgenic family carrying preprocecropin B and two families with porcine cecropin P1 cumulative mortality was observed to be 0-10% for five transgenic families with procecropin B and two families with cecropin B. When challenged with *V. anguillarum*, the cumulative mortality was 40% for non-transgenic control medaka, 20% in one transgenic family carrying preprocecropin B, between 20 to 30% in three transgenic families with procecropin B, and 10% in one family with porcine cecropin P1. RT-PCR analysis confirmed that transgenic fish from most of the F2 families expressed cecropin transgenes except those in three F2 families.

When comparing transgenic enhancement of disease resistance to that obtained via traditional selective breeding, we find that selection for body weight and disease resistance in salmonids has been particularly successful (Embody and Hayford 1925; Gjedrem 1979; Kincaid 1983). Already in 1925, Embody and Hayford selected surviving brook trout from a population with endemic furunculosis and increased the survival rate from 2 % in the initial population to 69% after three generations of selection. Ehlinger (1977) obtained reduced mortality rates due to furunculosis in brown trout and brook trout after selection. Okamoto et al. (1993) reported an infectious pancreatic necrosis (IPN) resistant strain of rainbow trout with average mortality of 4.3% compared with 96.1% in a highly sensitive strain. Bacterial disease resistance has been improved in channel catfish via both selection and intraspecific crossbreeding (Waters 2001; Padi

2003) and in some cases near total resistance was achieved. However, the selection and crossbreeding responses are inconsistent and sometimes non-existent is some strains, crosses, and lines.

Interspecific hybridization often results in fish with enhanced disease resistance. This is the case for ictalurid hybrids (Dunham et al. 2001). Some diploid salmonid hybrids are potentially valuable because of disease resistance inherited from the parent species that is usually not cultured, but these hybrids have low viability. The induction of triploidy can increase the hatchability of these potentially important hybrids (Parsons et al. 1986). Dorson et al. (1991) discussed hybridization attempts using coho salmon, which are considered to be resistant to a variety of salmonid viruses. In this example, disease resistance in the hybrids was improved, but overall viability was very poor. Viability was increased when hybridization was followed with triploidization, and Dorson et al. (1991) reported that the rainbow trout (*Oncorhynchus mykiss*) x char (*Salvelinus* spp.) triploid hybrids had general resistance to several pathogenic salmonid viruses (Dorson et al. 1991), but these triploid hybrids grew more slowly than diploids. Rainbow trout and coho salmon, *O. kisutch,* triploid hybrids also had increased resistance to infectious hematopoetic necrosis virus (IHN), but again, these hybrids grew more slowly than diploids (Dorson et al. 1991).

In general, the initial data on transgenic enhancement of disease resistance appears to indicate the potential of transgenesis to produce more effective and more consistent improvement compared to other biotechnologies. In some cases, including slower growth of hybrids, difficulty in producing hybrids, and negative correlated responses, utilizing other biotechnologies presents greater technology challenges than the transgenesis.

Colour

Three 'living color' fluorescent proteins, green fluorescent protein (GFP), yellow fluorescent protein (YFP), and red fluorescent protein (RFP or dsRed), have been expressed under a strong muscle-specific mylz2 promoter in stable lines of transgenic zebrafish, *Danio rerio* (Gong et al. 2002, 2003). These transgenic individuals exhibit vivid fluorescent colors (green, red, yellow, or orange) visible to unaided eyes under both daylight and ultraviolet light in the dark. These fish have been commercialized in Singapore, Japan, and the US and represent the first commercialization of transgenic fish and shellfish.

Biological reactors for pharmaceuticals

Transgenic fish might also be utilized as factories to produce medically important proteins. Wright and Pohajdak (2002) humanized fish insulin genes. Human insulin alpha and/or beta chains were produced using fish-preferred codons and regulatory sequences. The humanized genes were expressible in fish islet cells, and transgenic fish had islet cells containing these insulin genes and expressed these humanized insulin genes.

Theoretically, these islet cells (Brockmann Bodies) could be xenotransplanted into subjects having diabetes allowing normal glycemia to be achieved in the recipient of the islets.

Aquagene, Inc. also claims it has the ability to produce medical proteins in the liver of transgenic tilapia (Maclean et al. 2002). Potential advantages of the fish system are low cost, short generation interval, ease of culture, and reduced risk of prion contamination in comparison to mammalian systems.

Fish muscle may act as an efficient bioreactor for production of recombinant proteins. In the case of GFP/YFP and RFP transgenic zebrafish (Gong et al. 2003), the level of foreign protein expression was estimated between 3% and 17% of total muscle proteins, equivalent to 4.8-27.2 mg/g wet muscle tissue. The high level of foreign protein expression did not negatively affect the expression of endogenous mylz2 mRNAs.

The importance of domestication and strain selection in biotechnology

When wild fish are moved from the natural environment to the aquaculture environment, a new set of selective pressures are exerted on the population resulting in changes of gene frequencies and performance better suited for the aquaculture environment. This process of domestication occurs even without directed selection by the fish culturist. Domestication effects can be dramatic and can be observed in fish in as little as one-to-two generations after transplantation from the natural environment (Dunham 1996).

Usually, domesticated strains perform better in the aquaculture environment than wild strains. However, there are exceptions, and several examples exist for Nile tilapia, *O. niloticus*, and rohu, *L. rohita*, where wild strains grow better than domestic strains in the aquaculture environment. In many cases, there is a logical explanation for this exception primarily related to a lack of maintenance of genetic quality and genetic degradation. There is evidence that the poor performance of some domestic tilapia relative to wild conspecifics is related to poor performing founders, random genetic drift, inbreeding and introgression with slower growing species such as *Oreochromis Mossambicus* (Dunham et al. 2001). Additionally, many domestic strains have ancestry from one of the slowest growing wild/domestic strains of Nile tilapia from Ghana. Utilization of domestic strains instead of wild strains, and use of established, high-performance strains is the first step in applying genetic principles for improved fish culture management. Domestication has had and will continue to play a key role in the establishment and expansion of aquaculture production. Domestication and strain effects impact success of selection, intraspecific crossbreeding, interspecific hybridization, polyploidy, and sex reversal programs (Dunham et al. 2001; Dunham 2004).

Domestication is also important in transgenic growth responses, and Devlin et al. (2001) observed that salmonid GH gene constructs that have a dramatic effect on growth in wild rainbow trout strains (with naturally low growth rates) have little or no effect in strains

where growth rate has been enhanced by selection over many years. P1 and F1 rainbow trout derived from a slow growing wild strain and containing OnMTGH1 grew 17-fold faster than controls. Transgenic males and females eventually reached 8.2 and 14.2 kg compared to 0.220 and 0.171 kg for non-transgenic males and females, respectively. Maximum size of rainbow trout is near 20 kg. These wild transgenic rainbow trout grew no faster than a fast growing non-transgenic, domestic rainbow trout. This is indicative of the tremendous progress that domestication and selection has had on some aquaculture species and implies a possible 10 to 20-fold improvement of growth through selection.

Introduction of the transgene into the domesticated P1 only increased growth by 4.4% (Devlin et al. 2001); however, replication was extremely low and fast growing families could have been missed. This data indicates that the effects of selection and transgenesis are similar, but not additive in anyway in rainbow trout, but additive effects were observed for channel catfish (Dunham and Liu 2002) as GH transgenic catfish derived from domesticated and selectively-bred strains exhibited a moderate growth enhancement of 41% above that of select non-transgenic controls.

However, additional data on transgenic rainbow trout (Devlin et al. 2001) refutes this hypothesis concerning the effect of wild and domestic genetic backgrounds on response to GH transgene insertion. When OnMTGH1 was transferred to another wild rainbow trout strain, F77, growth was enhanced 7-fold which was almost 4-fold greater growth than that observed in a non-transgenic domestic rainbow trout. In this case, the wild transgenic is actually superior to the domestic selected strain indicating the genetic engineering can have a greater, rather than equivalent effect to the domestication and selection. Perhaps strain effects in general, epistasis, and genetic background are more significant in regards to affecting transgene response rather than the domestic or wild nature of the fish. When F77 was crossbred with the domestic strain, growth of the crossbreed was intermediate to the parent strains, a typical result (Dunham and Devlin 1998). However, the transgenic wild X domestic crossbreed was by far the largest genotype, 18-fold larger than the non-transgenic wild parent, 13-fold larger than the non-transgenic wild X domestic crossbreed, 9-fold larger than the non-transgenic domestic parent and more than 2.5-fold larger than the wild F77 transgenic (Devlin et al. 2001). The combined effects of transgenesis and crossbreeding had a much greater growth enhancement than crossbreeding or transgenesis alone. Additionally, a transgenic with 50% of its heritage from domestic sources was much larger than a wild transgenic, so great response from some types or forms of domestic genotypes is possible.

Pleiotropic effects of transgene insertion/ correlated responses to selection

Insertion of transgenes has the possibility of causing pleiotropic effects. The increased growth rate of the transgenic individuals could be a result of increased food consumption, feed conversion efficiency, or both. Fast growing transgenic common carp and channel catfish containing rainbow trout growth hormone gene had lower feed conversion efficiency than controls (Chatakondi 1995; Dunham and Liu 2002). Various transgenic

common carp families had increased, decreased, or no change in food consumption. Transgenic Nile tilapia also had a 20% improvement in feed conversion efficiency and were better utilizers of protein and energy compared to controls (Rahman et al. 2001). Transgenic tilapia expressing the tilapia GH cDNA under the control of human cytomegalovirus (hCMV) regulatory sequences exhibited about 3.6-fold less food consumption than nontransgenic controls, and food conversion efficiency was 2.9-fold better for the transgenic tilapia (Martinez et al. 2000). Efficiency of growth, synthesis retention, anabolic stimulation, and average protein synthesis were higher in transgenic than control tilapia. Martinez et al. (2000) observed differences in hepatic glucose, and in the level of enzymatic activities in target organs in the transgenic and control tilapia. A digestibility trial suggested that transgenic tilapia were more efficient utilizers of protein, dry matter, and energy (Rahman et al. 2001). Transgenic salmon converted feed about 10% more efficiently than controls, but had a huge increase feed consumption. This increase in growth due to a combination of both increases in feed consumption and feed conversion efficiency with feed consumption having a greater impact is almost universally observed in fish growth enhanced via selection, crossbreeding, hybridization, and other genetic biotechnologies (Dunham 1981; Al- Ahmad 1983; Dunham et al. 2001).

GH transgenic coho salmon, *O. kisutch*, had intestinal surface area 2.2 times that of control salmon and the growth rate was about twice that of controls. It seems likely that the enhanced intestinal surface area is a compensatory feature that is manifested in fast growing salmonids (Stevens and Devlin 2000b). The relative intestinal length was the same in transgenic and control salmon, but the surface area was greater for transgenics as a result of increased number of folds. This increase intestinal surface area was found in both Atlantic and coho salmon. This phenomenon has not been examined in fish growth enhanced by other genetic technologies.

The insertion of the rainbow trout growth hormone (rtGH) gene alters the survival of common carp (Chatakondi 1995). The number of F2 progeny inheriting this transgene was much less than expected. Differential mortality, a true pleiotropic effect, or loss of the recombinant gene during meiosis are likely explanations. These individuals were identified as transgenic or non-transgenic after reaching fingerling size. From that point on, survival of the remaining transgenic individuals was higher than that of controls when subjected to a series of stressors and pathogens such as low oxygen, anchor worms, *Lernia*, *Aeromonas,* and dropsy.

In general, positively correlated responses to selection have been associated with selection for body weight, including increased survival and disease resistance. There are, however, examples of long term selection programs for fish resulting in decreased bacterial disease resistance or survival possibly due to changes in genetic correlations or inbreeding. Increased fecundity, fry survival, and disease resistance were correlated responses to selection for increased body weight in channel catfish after one generation of selection for body weight (Dunham and Smitherman 1983; Smitherman and Dunham

1985). Disease resistance has both increased and decreased in lines of channel catfish selected for body weight for 6 generations (Padi 2003). In the case of salmonids, negative genetic correlations and correlated responses exist between resistances for some pathogens. Selection for growth had minimal effects on survival for tilapia. In several large scale genetic experiments, genetic correlations between growth rate and survival or survival for specific diseases have been estimated. Although the genetic correlation is not high, average close to 0.30, selection for rapid growth generally increases survival rate as a correlated response.

When subjected to low dissolved oxygen, 0.4 ppm, mean absolute survival was the same for transgenic and control common carp. However, when mean survival time was calculated for all fish dead or alive, the transgenic individuals had longer mean survival time than the non-transgenic full-siblings (Chatakondi 1995; Dunham et al. 2002b). Ventilation rate could be a possible explanation for the slightly better tolerance of low oxygen exhibited by the transgenic common carp. Transgenic channel catfish with the same rtGH construct as the common carp have a lower ventilation rate when subjected to low dissolved oxygen compared to controls. Also, growth hormone has a critical role in osmoregulation (Tang et al. 2001), and this could be related to response under conditions of oxygen stress.

Lines of channel catfish selected for increased body weight have the opposite reaction and a decrease in tolerance of low oxygen in comparison to randomly bred controls (Padi 1983). The tolerance of low dissolved oxygen is reduced in triploid fish compared to diploids (Dunham 2004).

GH transgenic fishes also exhibit body composition changes, but they are not as dramatic as those observed in mammals. However, the potential for similar dramatic body composition changes exist for transgenic fishes as the treatment of salmonids with sustained release recombinant porcine somatotropin decreased carcass fat by 42-50% (Mclean et al. 1994). Transgenic common carp had more protein, less fat, and less moisture than non-transgenic full-siblings (about a 10% change). Moisture content in GH transgenic Atlantic salmon was higher, relative to protein and ash, than in normal controls (Cook et al. 2000a).

Dunham et al. (2002c) examined body composition changes in GH transgenic common carp over 2 generations, F1 and F2. The carcass composition of transgenic muscle had a lower percentage of lipids and higher protein in both generations. Moisture was lower in F1 transgenic muscle, but unchanged in F2 transgenic individuals. The expression of the transgene had a significant effect in the proximate composition of transgenic fish. An increase in 7.5% protein and a 13% decrease in fat of a transgenic fish muscle results in superior quality of transgenic common carp muscle compared to control common carp muscle Transgenic channel catfish with the same rtGH cDNA also had more protein, less fat and less moisture in their edible muscle than non-transgenic full-siblings (about a 10% change). Transgenic *Oreochromis urolepis hornorum* containing one copy/cell of the

tilapia growth hormone (tiGH) cDNA under the regulatory sequences derived from the hCMV had lower levels of cholesterol, free alanine and aspartic acid in the muscle compared to controls (Martinez et al. 1999).

The increased level of protein in transgenic common carp and channel catfish muscle also results in increased levels of amino acids (Dunham 2003). However, amino acid ratios and fatty acid ratios were virtually identical in control and transgenic common carp and channel catfish, although some amino acids increase in proportion slightly more than others do.

GH transgenesis also affects muscle characteristics and activity. GH transgenic catfish had increased numbers of mitochondria in the cell, glycogen globules, and muscle fibers, but reduced number of fat globules (Dunham 2003). Muscle fiber size was unchanged. Perhaps due to these changes in amino acid levels and ratio, changes in fat and ultrastructure of the muscle, the flavor and texture (non-ingestion protocol) of transgenic catfish flesh was slightly better than non-transgenic controls.

Heterozygous growth hormone transgenic coho salmon had higher numbers of small-diameter fibers in somite muscles (Hill et al. 2000). Both the dorsal and lateral region of the somatic muscles were affected, suggesting that that the transgenic salmon grow by greater rates of hyperplasia relative to slower growing nontransgenic fish. Higher levels of activity were found for phosphofructokinase and cytochrome oxidase in white muscle of the transgenic fish, indicating a higher glycolytic and aerobic requirement in the muscle of transgenic fish. The GH gene insertion affected expression of several other genes, and many of the additional mRNAs in the transgenic fish were specifying myosin light chain 2, consistent with high level of expression in the early stages of muscle fiber construction.

Selection and crossbreeding for growth have no or little impact on body composition in fish (Rezk 1993). However, sex reversal and breeding and polyploidy have an impact on body composition, and flesh quality because of sexual dimorphism or in the case of polyploidy changes in body composition due to the alteration of sex hormone expression.

Zhu (1992) reported an increase in muscle thickness and body width in transgenic common carp, containing the human growth hormone gene. This observation was not quantified and was observed only in a few individuals. The effect of rtGH1cDNA (rainbow trout growth hormone cDNA) on body shape, dress-out yield and body composition were assessed in the F1 and F2 generations of transgenic common carp (Chatakondi et al. 1994, 1995; Dunham et al. 2002c). The correlation between head morphometric measurements and length or weight for F1 and F2 generations were negative (Chatakondi 1995) indicating that the fish's head does not grow proportionately to its length or weight. Various head, body, and caudal traits grew disproportionately faster than total body length and this effect was greater in transgenic fish in both generations compared to control common carp. The transgenic individuals have relatively

larger heads, deeper and wider bodies, and caudal areas compared to controls. Similar changes are seen in GH transgenic Nile tilapia as the head: total length ratio, viscera-somatic index, and hepato-somatic index increased in transgenic fish relative to controls (Rahman et al. 2001).

The condition factor (K), was proportionately higher in transgenic common carp in most of the families (Chatakondi 1995). However, families 1 and 7 of F1 generation and 69 and 70 of F2 generation had a lower condition factor than their controls despite higher weight increase. The altered body shape of transgenic fish resulted in improved dressing percentage in the F2 generation, and a similar result was obtained for transgenic channel catfish containing the same GH construct.

Transgenic wild-strain rainbow trout had the slender-body shape similar to that of wild controls, but their final size at sexual maturity was much larger than non-transgenic wild rainbow trout (Devlin et al. 2001), thus no pleiotropic effect on body shape was seen for these fish in contrast to the stockier more truncate body shape of GH transgenic domestic common carp compared to non-transgenics. However, the domestic transgenic rainbow trout derived from a deep-bodied strain, despite their minimal growth enhancement, had an even deeper body depth than the controls caused by either increased muscle or tremendous visceral fat deposits or both.

Selection for body weight did not affect dressout percentage, visceral percentage, head percentage, or seinability (Dunham 1981). Three and four generations of selection for increased body weight resulted in increased dressout percentage, and no change in body composition and seinability (Rezk 1993; Padi 1995).

Change in body shape as a result of GH gene transfer is common in transgenic fish. The P1 generation of transgenic Pacific salmon, containing chinook salmon growth hormone gene had an impressive growth rate with a slightly lower condition factor (Devlin et al. 1995a). However, the excessive levels of growth hormone resulted in morphological abnormalities in head, fin, jaw, and operculum as a result of excessive cartilage and bone growth of the fastest growing transgenic fish. Insertion of a sockey salmon metallothionein promoter (pOnMTGH1) gene construct into coho salmon altered centroid size, and the dorsal caudal peduncle and abdominal regions were also distinctly enhanced in transgenic fish when compared to controls (Ostenfeld et al. 1998). Morphological changes of both whole body and syncranium were prominent.

GH gene transgenesis also affects gill morphology. Transgenic Atlantic salmon (Stevens and Sutterlin 1999) and Pacific salmon (Stevens and Devlin 2000a) had different gill morphology than controls, but the difference was expressed in different ways in the 2 species. Transgenic Pacific salmon had gill filaments similar to controls in length but had smaller lamellar spacing. Atlantic transgenics had longer gill filaments than controls but with similar lamellar spacing to controls. This illustrates that the pleiotropic effects from GH transgenesis can be dissimilar for even closely related species.

The largest of P1 transgenic salmon produce offspring with extraordinary (30-fold) growth. Unfortunately, these fish are subviable and virtually all die. The endocrine stimulation has been elevated to pathological levels in these GH transgenic salmon, and excessive, deleterious deposition of cartilage was observed (Devlin et al. 1995ab). This cartilage deposition is analogous to the mammalian acromegaly syndrome. This effect can be sufficiently severe in that impaired feeding and respiration may result in reduced growth and poor viability. Consequently, salmon that ultimately display the greatest growth enhancement as adults are those that have been only moderately stimulated (Devlin et al. 1995a,b). Progeny from transgenic parents with more moderate accelerated growth do not exhibit reduced survival and increased skeletal anomalies.

GH transgenic rainbow trout also exhibited cranial deformities (Devlin et al. 2001). Despite their minimal growth enhancement, domestic transgenic rainbow trout exhibited cranial deformities. Additionally, domestic rainbow trout receiving exogenous growth hormone showed modest increases in growth, 9%, but also had cranial abnormalities and silver body coloration, whereas controls did not have these characteristics (Maclean et al. 1997).

The deformities could be a species-specific phenomenon. Despite much more significant growth acceleration compared to the slow growing rainbow trout GH transgenics, P1, F1, F2, F3 and F4 GH transgenic common carp and channel catfish do not exhibit deformities. Additionally, no abnormalities were apparent in rapidly growing GH transgenic Nile tilapia, although minor changes to skull shape were observed in some fish (Rahman et al. 1998).

Not only the mean phenotypic change, but also the range of phenotypic variation needs to be assessed as a consequence of foreign gene integration in the fish genome. The transgenic common and silver crucian carp had a coefficient of variation twice that of non-transgenic controls (Chen et al. 1990). This is in contrast to the results of Zhang et al. (1990), and may be due to the less consistent expression of the transgenic carp in the experiments of Chen et al. (1990). Zhang et al. (1990) observed that the smallest and largest GH transgenic common carp were larger than the corresponding smallest and largest full-sibling control common carp in the F1 generation. The coefficient of variation for body weights was smaller for transgenic fish than non-transgenic fish in the families in which the mean body weight of the transgenic common carp was larger than the control common carp. The growth response was variable in transgenic and non-transgenic common carp when analyzed by the two generations, by sex, by age and environments.

Reproductive traits have not been greatly affected by GH transgenesis (Dunham 2004). Fecundity is not affected by insertion of rainbow trout GH in common carp. Precocious sexual development was not observed in transgenic common carp. However, GH transgenic male tilapia had reduced sperm production. Female GH transgenic Nile tilapia had a lower gonadosomatic index than non-transgenic siblings in both mixed and separate

culture conditions (Rahman et al. 2001). Transgenic male gonadosomatic index was higher in mixed culture and lower in separate culture than that of their non-transgenic siblings.

Correlated responses to selection, like transgenesis, have had minimal impact on reproduction (Dunham et al. 2001). However, selection, intraspecific crossbreeding, sex reversal, and polyploidy can be directly used to significantly increase reproduction in some cases and decrease it in others.

GH transgenic coho salmon exhibit a colour change (Devlin et al. 1995b; Devlin 1997). Individuals containing opAFP or OnMT salmon GH constructs have lighter skin pigmentation. This is a reliable marker to identify transgenic salmon prior to first feeding (Devlin et al. 1995b). Control fish possessed the normal brown coloration typical of coho salmon alevins, whereas the GH transgenics had a distinct green coloration.

The most important pleiotropic effect, which is one of the major explanations for the growth differences in transgenic and control salmon, is the accelerated smoltification of the transgenics. The transgenics smolt up to two years early and display enhanced silver coloration and osmoregulatory ability (Devlin 1997).

Just as GH gene transfer affects multiple traits, interspecific hybridization, polyploidy, and sex reversal often affect a few to several traits. The hybrid between the channel catfish female and the blue catfish, *Ictalurus furcatus*, male exhibits heterosis for several traits, and is the only one of 28 catfish hybrids evaluated exhibiting over dominance for economic traits (Smitherman and Dunham 1985). The channel-blue hybrid has increased growth, growth uniformity, disease resistance, tolerance of low oxygen, dressing percentage, and harvestability.

Sometimes an interspecific hybrid does not exhibit heterosis for any traits, but is still quite important for aquaculture application as it expresses a good combination of beneficial traits from both parent species. Examples of this phenomenon include a hybrid between the two walking catfish *Clarias macrocephalus* and *C. gariepinus,* the bass hybrid between *Morone saxatilis* and *Morone chrysops* and the interspecific tilapia hybrids such as *O. niloticus* x *O. aureus*. The main catfish cultured in Thailand is a cross between African, (*C. gariepinus*) and Thai (*C. macrocephalus*) catfish. This cross combines fast growth rate of the African catfish with the desirable flesh characters of the Thai catfish (Nwadukwe, 1995). The overall product is improved and the flesh is still acceptable to Thai consumers, although it does not grow as fast as the pure African catfish. The rohu x catla hybrid grows almost as fast as pure catla, but has the small head of the rohu, and is therefore useful in Indian aquaculture (Reddy 1999). *Catla catla* x *Labeo fimbriatus* hybrids were reported to have small heads of *L. fimbriatus* and deep body and nearly equal growth rate to the catla. Dressing percentage was also improved in this hybrid (Basavaraju et al. 1995). The sunshine bass hybrid (white bass x striped bass) has a suite of advantageous traits including, good osmoregulation, high thermal tolerance,

resistance to stress and disease, high survival in culture and modified water bodies, fast growth in aquaculture conditions, and ability to utilize soy beans as a protein source compared to its parent species (Hodson et al. 1987).

Triploidy can also affect multiple traits (Dunham 2004). Triploid fish are generally sterile, females have greatly reduced production of sex hormones, but triploid males can develop secondary sexual characteristics, exhibit spawning behavior and induce females to expel eggs even though they are unable to fertilize them. Both triploidy and sex reversal and breeding can positively affect flesh quality.

Combining genetic enhancement programs

The ultimate aquaculture genotypes will likely be produced by combining various genetic enhancement programs to improve suites of traits. Crossbreeding can improve performance of genetically male tilapia, GMT (Mair and Abucay 2001). Rotational mating of five strains of pure *O. niloticus* have been used with combined within family selection for growth and progeny testing selection for GMT sex ratio (% male). After 3 generations of selection for growth and two for sex ratio, preliminary results indicate a significant response to selection. Combining selection with crossbreeding and selection with hybridization can promote growth better than using these programs singly for species such as catfish and carp (Dunham et al. 2001).

In Israel, Hinits and Moav (1999) were able to improve common carp growth more by using genetic engineering with crossbreeding than by using crossbreeding alone. Similarly, when salmon metallothionein promoter/salmon GH1 cDNA, OnMTGH1, was transferred to the wild coho salmon, F77, growth was enhanced 7-fold which was almost 4-fold more than a domestic salmon (Devlin et al. 2001). When F77 was crossbred with the domestic strain, growth of the crossbreed was intermediate to the parent strains, a typical result (Dunham and Devlin 1998). However, the transgenic wild X domestic crossbreed was by far the largest genotype, 18-fold larger than the non-transgenic wild parent, 13-fold larger than the non-transgenic wild X domestic crossbreed, 9-fold larger than the non-transgenic domestic parent and more than 2.5-fold larger than the wild F77 transgenic (Devlin et al. 2001). The combined effects of transgenesis and crossbreeding had a much greater growth enhancement than crossbreeding or transgenesis alone.

Channel catfish transgenic for rainbow trout GH exhibited a moderate growth enhancement, 41%, and were derived from domestic, selectively bred catfish. If we extrapolate from a series of experiments starting with slow-growing wild strains of channel catfish and then improve their growth through domestication (Dunham 1996), followed by further improvement from selective breeding (Padi 1995), growth in ictalurid catfish has been improved by about 10-fold. This result is similar to those for transgenic salmon.

Environmental risks and fitness traits

Commercialization of transgenic aquatic organisms on a large scale may have a variety of ecological implications (Hallerman and Kapuscinski 1992a,b, 1993). Eventual escape of transgenic aquatic organisms from confinement will occur from a commercial facility, and the range of receiving ecosystems is broad.

Most data indicate that wild fish are more competitive than domestic fish (Dunham 1996), resulting in the elimination of the domestic fish and their potential positive or negative impacts. However, recent evidence from salmonid research indicates that there are situations where domestic fish can have genetic impact on wild populations. When repeated large-scale escapes of domestic fish occur, genetic impact can occur just from the swamping effect of sheer force of numbers. Transgenic fish could make an impact in this scenario, but again the consequences should not vary much from that of fish genetically altered by other means.

All available data except that for GH transgenic medaka grown in aquaria indicates that transgenic fish are less fit than non-transgenic fish, and would likely have little if any environmental impact. Additionally, domesticated transgenic fish would be expected to have less environmental risk than wild transgenic fish based on the discussion above. However, the greatest environmental risk that a transgenic fish would have is when the gene insert would allow the transgenic genotype to expand its geographic range, essentially becoming equivalent to an exotic species. About 1% of such releases of exotics result in adverse environmental consequences as not all releases result in establishment of populations, and some do not result in measurable environmental impact (Dunham 1996). However, when environmental impact occurs, it can be devastating as was the case with the Nile perch.

Altering temperature or salinity tolerance would be analogous to the development of an exotic species since this would allow the expansion of a species outside its natural range. This type of transgenic research and application should be avoided. Antifreeze protein genes from winter flounder have been introduced into Atlantic salmon in an attempt to increase their cold tolerance (Shears et al. 1991). If this research were successful, a real possibility of environmental impact exists. Similarly, if tilapia were made more cold tolerant a strong possibility of detrimental environmental impact exists. Sterilization could reduce risk, but genetic means of sterilization such as triploidy decrease performance (Dunham 1996). Additionally, fertile broodstock are necessary, so risk is minimized but not eliminated. Transgenic sterilization, to be discussed later, is potentially a much better option than triploidy.

Efforts should be organized to evaluate the potential environmental risk of transgenic fish. The reproductive performance, foraging ability, and predator avoidance are key factors determining fitness of transgenic fish, and should be a standard measurement prior to commercial application. This type of data measurement is similar to the net-

fitness methodology proposed by Muir and Howard (2001, 2002). Most ecological data on transgenic fish gathered to date indicate a low probability of environmental impact. Extremely fast growing salmon and loach have low fitness and die (Devlin et al. 1994, 1995a,b).

Several models have been developed that estimate the genetic risk of transgenic fishes. Muir and Howard (1999) evaluated a model and described the Trojan gene effect, the extinction of a population due to mating preferences for large transgenic males with reduced fitness. Therefore, reduced fitness as well as increased fitness has potential adverse ecological effects. This modeling was based on experimental results of medaka in aquaria.

Hedrick (2001) developed a deterministic model indicating that if the wild-type male has a mating advantage, relative to the transgenic line, and there is a general viability disadvantage associated with the transgene (analogous to the Trojan gene effect of Muir and Howard (1999)), then for 66.7% of the possible combinations the transgene increases in frequency. For 50% of the combinations, the possible combinations of the mating and viability parameters, the transgene goes to fixation. The increase in the frequency of the transgene reduces the viability of the natural population, increasing the probability of extinction of the natural population.

In another modeling exercise, Muir and Howard (2001) again conclude that a transgene is able to spread to a wild population even if the gene markedly reduces a component of fitness based on data from a laboratory population of medaka harboring a regulatory sequence from salmon fused to the coding sequence for human growth hormone. The juvenile survival of transgenics was reduced in the laboratory but growth rate increased, resulting in changes in the development rate and size-dependent female fecundity. The important factors in the model were the probabilities of the various genotypes mating, the number of eggs produced by each female genotype, the probability that the eggs will be fertilized by the sperm of each male genotype (male fertility), the probability that an embryo will be a specific genotype given its parental genotypes, the probability that the fry will survive and parental survival. Muir and Howard (2001) indicate that transgenes would increase in populations despite high juvenile viability costs if transgenes also had sufficiently high positive effects on other fitness traits. Sensitivity analyses indicated that transgene effects on age at sexual maturity should have the greatest impact on transgene allele frequency. Juvenile viability had the second greatest impact. A weakness in the simulation was the fact that data on the effect of predation in the wild were not utilized in the model, biasing viability estimates (Muir and Howard 2001). Other considerations are that the environment was artificial. The mating preference does not exist for many fish including catfish. Data were not entered in the models to account for genotype-environment interactions, which are likely. Predation is absent as Muir and Howard (2001) indicate and the overall performance of the fish was not accounted for.

Body size does not necessarily result in mating advantages. Rakitin et al. (2001) utilized allozymes and minisatellites to determine that male size, condition factor, and total or relative body-weight loss over the season were not correlated with the estimated proportion of larvae sired by each Atlantic cod male during the spawning season. Similar results were observed in salmon (Doyle 2003). However, Atlantic cod male reproductive success was affected by female size, with males larger (>25% total length) than females siring a smaller proportion of larvae (Rakitin et al. 2001). In this case, large size was reproductively disadvantageous. Transgenic rainbow trout experienced early maturation at 2 years of age, but in the same season as the controls, a potential advantage for the transgenics. However, spawning outside the normal spawning season can also be a disadvantage potentially subjecting embryos and larvae to adverse/lethal weather conditions and inadequate forage.

Although there may be cases where size increases reproductive fitness of both sexes, cultured transgenic fish may not be allowed to grow large enough to have a mating advantage as escapees (Doyle 2003), and data for transgenics of larger aquaculture species have not shown any reproductive advantage for the transgenics. Fast growing transgenic tilapia have reduced sperm production. Transgenic channel catfish and common carp have similar reproduction and rate of sexual maturity compared to controls (Dunham et al. 1992; Chen et al. 1993; Chatakondi 1995). Spawning success of transgenic channel catfish and controls appeared similar. When the two genotypes were give a choice in a mixed pond the mating was at random, and spawning ability of transgenic and control channel catfish was equal (Dunham et al. 1995).

Genotype-environment interactions are important and occur for growth of transgenic channel catfish (Dunham et al. 1995). Transgenic channel catfish containing salmonid growth hormone genes grew 33% faster than normal channel catfish in aquaculture conditions with supplemental feeding. However, there was no significant difference in growth performance between transgenic and non-transgenic channel catfish in ponds without supplemental feeding indicating equal foraging abilities, and the inability of transgenic catfish to exhibit their growth potential with limited feed (Chitmanat 1996). Foraging ability of transgenic and control catfish is similar under these conditions of competition and natural food sources, and growth is not different between transgenic and control catfish in these more natural conditions. When grown under natural conditions where food is limiting, the transgenic channel catfish has slightly lower survival than controls and grows at the same rate as non-transgenic controls. As in the case of most genetic improvement programs, genetically altered fish need adequate food to express their potential. Genotype-environment interactions should be examined for other types of transgenic fish.

The faster growing transgenic fish could have impaired swimming leading to predator vulnerability, problems in capturing prey, reduced mating ability for some species, and reduction in competitiveness for any trait requiring speed. Selection for swimming ability

may be one of the primary mechanisms limiting the genetic increase in size of fish and preventing fish from evolving to larger and larger sizes.

This concept might be illustrated by comparing geographic strains of silversides, *Menidia menidia.* Those from Nova Scotia ate more food, had more efficient feed conversion, and grew faster than a population from South Carolina (Billerbeck et al. 2000). Nova Scotia strain was more vulnerable to predation than South Carolina strain, and predation increased with growth rate and feeding rate both within and between strains (Billerbeck et al. 2001). Maximizing energy intake and growth rate can engender fitness costs in the form of increased vulnerability to predation (Doyle 2003).

Predator avoidance was slightly better for non-transgenic catfish fry and fingerlings when exposed to largemouth bass, *Micropterus salmoides*, and green sunfish, *Lepomis cyanellus*, than transgenic channel catfish (Dunham 1995; Dunham et al. 1995; Dunham et al. 1999) GH transgenic salmon have an increased need for dissolved oxygen (Stevens et al. 1998; Cook et al. 2000b,c), have reduced swimming ability (Farrell et al.1997; Stevens et al. 1998) and lack of fear of natural predators (Abrahams and Sutterlin 1999).

On an absolute speed basis, transgenic coho salmon swam no faster at their critical swimming speed than smaller non-transgenic controls, and much slower than older non-transgenic controls of the same size (Farrell et al. 1997). Ostenfeld et al. (1998) indicate that coho salmon containing pOnMTGH1 had altered body contour, centroid size, enhanced caudal peduncle, and enhanced abdominal regions compared to controls. The most prominent alterations were the change in the syncranium and the head of the transgenics was less elliptical. The overall body shape is less fusiform for the transgenic coho salmon. Therefore, the decrease in swimming ability may be a result of loss of hydrodynamics and increased drag coefficients caused by the altered body shape. This change in body shape might also alter leverage or efficiency of the muscle movements for swimming. The inferior swimming ability of the transgenic salmon should cause them to have inferior predator avoidance, inferior ability to capture food, and inferior ability to migrate to reach the sea or return to reproduce.

Transgenic fish could be more competitive in seeking feed. Devlin et al. (1999) examined the ability of F1 coho salmon (250 g) containing a sockeye metallothionein-B promoter fused to the type 1 growth gene-coding region to compete for food through higher feeding motivation. The transgenic coho salmon consumed 2.5 times more contested pellets than the controls and 2.9 times more pellets that the non-transgenic controls, indicating a high feeding motivation of the transgenic fish throughout the feeding trials. The shortcomings are that this is a highly artificial environment and a food type that will not be encountered under natural conditions.

F2 transgenic Atlantic salmon contained a salmon growth hormone gene that was continuously expressed in the liver, enhancing grow 2.62 to 2.85-fold over the size range 8-55 g and improving feed conversion efficiency by 10% (Cook et al. 2000a). However,

these transgenic fish had higher metabolic rates. Atlantic salmon, and starved transgenic Atlantic salmon also lost protein, dry matter, lipid, and energy more quickly than controls, which could be a definite disadvantage in an environment with limiting food.

Lee et al. (2003) looked at a combination of the starvation and swimming ability in adult GH transgenic coho salmon. Routine oxygen consumption was 35% higher in 1 day starved and 21% higher in 4 day starved adult transgenic coho salmon relative to end of migration ocean-ranched coho salmon. Critical swimming speed was lower in 4 day starved transgenic coho salmon. Transgenic individuals swam energetically less efficiently than ocean-ranched fish, and excess post-exercise oxygen consumption measured during the first 20 minutes of recovery was higher in transgenic coho salmon compared with ocean-ranched coho salmon, which had a faster rate of recovery.

As expected fluorescent transgenic fish have no advantage in survival and reproduction compared to the wild-type fish (Gong et al. 2003). Because of their unnaturally bright color, these transgenic fish would be expected to have an increased vulnerability to predation.

All transgenic fish evaluated to date (other than the medaka) have fitness traits that are either the same or weaker compared to controls. The increased vulnerability to predators, lack of increased growth when foraging, and unchanged spawning percentage of these transgenic fish examples indicate that some transgenic fish may not compete well under natural conditions, or cause major ecological or environmental damage. Although transgenic fish may be released to nature by accident, ecological effects should be unlikely because of these examples of reduced fitness; however additional evaluation is needed.

Genetic sterilization

One method to reduce environmental risk is to genetically sterilize the production fish. The best technology currently available to accomplish this is triploidy. However, it has several disadvantages. Triploid induction is not commercially feasible for all species, it is sometimes not 100% effective, it requires fertile, diploid brood stock and triploidy has adverse effects on some economic traits partially negating some of the improved performance of the transgenic genotype.

For example, resistance to the bacterial pathogen *Vibrio anguillarum* was not affected in transgenic diploid and triploid coho salmon transgenic for growth hormone fish relative to their non-transgenic counterparts when they were infected at the fry stage, but was lower in transgenic fish when infected near smolting (Jhingan et al. 2003). Vaccination against vibriosis provided equal protection to both transgenic and non-transgenic fish. However, the triploid fish, transgenic and non-transgenic, showed a lower resistance to vibriosis than their diploid counterparts. Triploid GH tilapia have reduced growth compared to diploid GH tilapia (Maclean et al. 2002).

Sex reversal and breeding to develop transgenic lines that are genetically monosex would be a feasible method to genetically sterilize exotic species of fish. However, these lines could only be used in environments where individuals of the opposite sexual genotype of the same species were not in the same watershed or aquatic environment, and for absolute reproductive confinement, only in species that have no polygenic sex determination.

Transgenic sterilization has the potential to render transgenic fish sterile without the drawbacks of polyploidy and sex reversal. Transgenic sterilization would almost completely eliminate environmental risk and may be the most important key for commercialization of transgenic fish. Still some will argue that the potential would exist for escaped transgenically sterile fish to disrupt mating of wild conspecifics, thus potentially reducing population numbers. Massive escapement could lead to this scenario. Unless repeated large scale escapement occurs, this potential effect would be temporary. Perfect confinement is not possible for all applications of transgenic fish. However, the combination of drastically reduced fitness of domestic transgenic fish, genetic sterilization, transfer of appropriate gene constructs, and appropriate physical confinement will reduce risk to such negligible levels that the benefits will be much greater than the risks.

Preliminary results where the GnRH antisense approach was utilized to transgenically sterilize fish has been very promising. Antisense is most effective when rare messages are targeted and the antisense construct is driven by a strong promoter. Carp beta actin-tilapia salmon type GnRH antisense construct was injected into Nile tilapia (Norman Maclean, personal communication; Maclean et al. 2002). Transgenic females were crossed with wild type males. A reduction in fertility of about half that of non-transgenic control females was observed. Fertility was much more greatly reduced in transgenic males crossed to control females. In some cases, 0% fertility was obtained with an average about an 80% reduction in fertility. Limited data on transgenic females crossed with transgenic males indicated near zero fertility.

Tilapia beta actin-tilapia sea bream GnRH antisense construct was injected into Nile tilapia. In this case, no reduction in fertility of heterozygous transgenic males and females was observed.

Limited data on transgenic females crossed with transgenic males indicated no reduction in fertility. Reciprocal crosses between sea bream and salmon GnRH antisense transgenics gave hatch rates that appeared to be dictated by the salmon GnRH antisense parent. Apparently, salmon type GnRH has a more critical role in fertility than sea bream type GnRH.

This type of result has been confirmed in transgenic rainbow trout. Transgenic rainbow trout containing salmon type antisense GnRH from Atlantic salmon, *Salmo salar*, driven by either the GnRH or histone 3 promoter had reduced levels of GnRH and appeared to

be sterile (Uzbekova et al. 2000a,b). Preliminary data indicated that spermiation of transgenic males was only obtained after prolonged treatment with salmon pituitary extract, whereas control males spermiated naturally. Data are still needed for the females.

Another strategy would be to utilize multiple Sterile Feral constructs for redundant sterility, virtually ensuring no escapement (Thresher et al. 2001). This technology also allows protection of proprietary germplasm. With this approach, embryonic development is disrupted whenever a transgenic individual is mated with any genotype, transgenic or non-transgenic. Embryos can only survive in an aquaculture setting, where the culturist adds a compound to the hatching water to block the action of the embryonic disruption construct, which is some type of gene knock out mechanism.

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Participant Discussions

Muir: I am interested in the conclusions regarding conventional breeding. One of the first transgenics was named after super mouse. The chicken was one of the next transgenic organisms after the mouse, and no growth effect was seen. The transgenic pig was found to get ulcers and skin disease. In Bob's results, domesticated salmon or trout with growth hormone show very little enhancement. Growth is receptor limited, and if saturated, there should be no further increases in growth. If one tries to augment further, you won't see growth effects beyond conventional breeding.

Dunham: Some of the initial data would lead you down that path. Of course, with catfish and carp we do see some augmentation. If you were a big commercial grower, getting a 50% increase in growth rate would make you do hand stands. The loach they are using in Korea are wild, where they also get very high growth responses. Perhaps there is something in Bob's paper that I've misinterpreted; in his initial work he is not getting much of a response, maybe twenty to thirty percent in domestic trout. However, in one of the later papers, the greatest enhancement was in a domestic/wild crossbreed transgenic trout. Even though this fish was half domestic, it was growing 17 times faster. In contrast to 4-fold increase in a wild transgenic. Again, Bob will correct me if I miss something in that

paper. Even with a significant domestic genome, there is still the possibility for a great enhancement.

Devlin: The results differed depending on the species. Limited growth enhancement was seen with highly domesticated rainbow trout. The domestic and wild rainbow trout strains were picked for their extremes. Where we do see growth enhancement combining transgenesis and domestication is with partially domesticated coho salmon, which have been selected for only 3-5 generations or so. In that case, systems perhaps are being geared up, maybe receptor levels or metabolic pathways are increased. Ultimately after 100 years of selection for growth in trout, the sensitivity to GH in the growth pathway may have been reduced.

Kapuscinski: Do you feel the data out there were good enough to unequivocally say that the transgenic lines are less likely to avoid predators? My reading of your paper is that it's a good first step but it needs to be repeated under a different experimental design and statistical analysis. I'm not sure if we can pool data from different ponds since you had different starting conditions. If I had to make a statement I'd say it's still an open question. We need to do much more rigorously designed experiments.

Dunham: I didn't make the statement that the data was unequivocal. There is definitely the need for more well done experiments and more data. I would disagree that the paper in Marine Biotech was not well done (8 replicates is enough). It wasn't an overly dramatic result. Consistently, under natural conditions (plankton, hiding places, natural predators), pond after pond there was a lower survival of the transgenics vs. the control. More dramatically done by Bob, the transgenic salmon have a much less ability to avoid predators than controls. After exposure to a heron model, they become less wary of a predator more rapidly than controls. The two or three experiments that have been done have been good ones and point towards GH GM fish having lower predator avoidance.

Maclean: I'm a little worried we might get hung up on predator avoidance. I think it's a little simplistic that it's going to be naturally uniform. It's a behavior affected by numerous parameters. Don't be simplistic. The same is true for growth. Growth is just not a response to growth hormone. All of these are quite complex parameters.

Unknown: There was a paper published on cloned animals recently describing behavioral changes (changes in behavior less aggressive). Discussing this with pathologists, they will say it's the amount of attention they receive. Between an animal conventionally bred and raised on a farm, and the GM

animal, the GM animal receives more human attention. This causes less animal to animal bonding which could affect their behaviour.

THE SCIENCE AND BUSINESS OF TRANSGENIC SALMON: PROMISES AND PROBLEMS

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Abstract

Stable lines of transgenic Atlantic salmon possessing either antifreeze protein (AFP) genes or a salmon growth hormone (GH) gene construct have been generated over the past 20 years. The AFP gene transfer studies were initiated in 1982. The AFP transgene integrated into the genomic DNA with AFP being found in the blood of all 5 generations to date. However, antifreeze protein levels were low and a means to raise these levels needs to be developed. Our GH gene transfer studies were initiated in 1989. Evidence to date indicates that a single copy of the GH transgene was integrated into chromosomal DNA and passed on to successive generations in a Mendelian fashion. Laboratory studies indicate that our GH transgene enhances growth rates to the point where Atlantic salmon can reach market size (4-6 kg) a year earlier than can non-transgenics cultured commercially in Atlantic Canada. The GH gene transfer technology was patented, licenced to Aqua Bounty Farms Inc. The GH transgenic salmon are currently under, or being prepared for, review by regulatory authorities in United States and Canada for use in commercial aquaculture ventures. This communication centres around our experience with Atlantic salmon and outlines our plans and progress towards demonstrating the safety of transgenic fish to the consumer.

Résumé

Des souches stables de saumons atlantiques transgéniques qui possèdent soit un gène d'une protéine antigel (AFP) ou un gène hybride de l'hormone de croissance (GH) du saumon ont été produites au cours des vingt dernières années. Les études sur le transfert du gène AFP ont été entreprises en 1982. Le transgène AFP a été intégré dans l'ADN génomique de saumons, et la protéine antigel est présente dans le sang des cinq générations produites à ce jour. La teneur en protéine antigel est cependant faible, et un moyen d'accroître cette teneur doit être élaboré. Nous avons entrepris nos études sur le transfert d'un gène GH en 1989. Les données obtenues à ce jour indiquent qu'un seul exemplaire du transgène GH a été intégré dans l'ADN chromosomique puis transmis d'une génération à l'autre selon les lois mendéliennes. Les résultats d'études menées en laboratoire indiquent que notre transgène GH améliore le taux de croissance au point où le saumon atlantique transgénique peut atteindre la taille marchande (de 4 à 6 kg) en une année de moins que le saumon non transgénique élevé à des fins commerciales au Canada atlantique. La technologie de transfert du gène GH a été brevetée, et Aqua Bounty Farms

Inc. est l'entreprise qui détient la licence relative à cette technologie. Les saumons dotés du transgène GH font présentement, ou feront bientôt, l'objet d'un examen par des organismes de réglementation aux États-Unis et au Canada aux fins d'exploitation en aquaculture commerciale. Le présent document est axé sur notre expérience en rapport avec le saumon atlantique et présente nos plans et les progrès que nous avons réalisés en matière de démonstration de la salubrité des poissons transgéniques pour la consommation.

Introduction

World fish stocks are severely damaged. The vast majority are exploited to the maximum extent or over-fished, and many are in danger of commercial extinction (Pauly et al. 1998; Watson and Pauly 2001; Myers and Worm 2003). As the world population continues to grow exponentially, it is clear that if fish are to maintain their current status as an essential food resource, production must be dramatically improved. Aquaculture stands alone as the only sustainable means of meeting demands for fish in the future (New 1997).

A key element to enhanced production of cultured species is the development of genetically superior broodstocks that are tailored to their culture conditions and to the market. Characteristics that are generally desirable include improvements in growth rates, feed conversion efficiencies, disease resistance, cold and freeze resistance, tolerance to low oxygen levels, and the ability to utilize low cost, or non-animal protein diets (Hew and Fletcher 1997).

Aquaculture is still in its infancy relative to the farming of terrestrial livestock. Despite the acknowledged power of traditional selection and breeding methods, the development of superior broodstock using this process is still relatively slow, and while such broodstock development programs have been underway for salmon since 1971 (Gjedrem 1997), many aquaculture ventures are still reliant on broodstock fish collected from the wild. If we are to realize the increased production needed to meet the requirements of the 21st century, a quantum leap in broodstock development is needed.

Transgenic technology provides a means by which such a quantum leap in production is possible (Fletcher and Davies 1991; Hew and Fletcher 2001; Melamed et al. 2002). The identification, isolation, and reconstruction of genes responsible for desirable traits, and their transfer to broodstock, offer powerful methods of genetic/phenotypic improvement that would be difficult, if not impossible, to achieve in fish using traditional selection and breeding techniques (Devlin 1997).

This brief communication highlights our experiences on the road towards generating transgenically enhanced Atlantic salmon broodstocks for commercial aquaculture. The issues involved in the production of transgenic fish and their successful integration into

the aquaculture industry involve not only science but also food safety, environmental risk assessment, animal welfare, producer acceptance, consumer acceptance, and intellectual property protection (Fletcher et al. 1999a).

Transgenic salmon

We came into the field of transgenics some twenty years ago in response to problems faced by the aquaculture industry along the east coast of Canada where most of the coastal waters are characterised by ice and sub-zero temperatures (0 to -1.8°C) during the winter. This environment is lethal to most teleost fishes with the primary exceptions being those fish that can produce antifreeze proteins (Fletcher et al. 2001). Salmonids and many other commercially important fish cannot synthesize antifreeze proteins. Thus, although many locations in eastern Canada appear suitable for salmon grow-out operations, most of them cannot be used with existing technology because temperatures drop below -0.7°C (Rosenthal et al. 1995).

Because of these environmental factors, the marine cage culture of salmonids in eastern Canada is almost entirely restricted to a relatively small area in the most southerly part of the region where the waters freeze infrequently (Aiken 1986; Hew et al. 1995). However even at this location where the annual value of the industry is approximately $160 million, the danger that water temperatures could decline to lethal levels remains, and mortalities attributed to super chill are not uncommon. During the winter of 2003 losses were estimated at $12 million CDN (Raynor and Campbell 2003). The potential danger of such losses severely restricts the development of aquaculture along the east coast of Canada, and along the coast of Maine, USA.

In addition to the potential dangers from super chill, low, non lethal water temperatures result in slow growth rates and in some species, reduced disease resistance. For example, Rodrigues et al. (1998) found that in carp the major histocompatability (MHC) genes were down-regulated at low temperatures, effectively preventing the fish from mounting an immune response to viruses. Similar studies are being carried out on salmonids (Brian Dixon, personal communication). Clearly, the problems (slow growth, reduced disease resistance, and danger from freezing) associated with economically successful culture of salmon in cold, and at times icy, water is considerable. The challenge for the scientific community is to find a means of producing strains of salmon that perform well under these conditions and thus facilitate the expansion of aquaculture and economic development throughout the entire Atlantic coastal region (Fletcher et al. 1999b). A potential solution to these problems became evident when Palmiter et al. (1982) and Hammer et al. (1985) demonstrated the power of transgenic technology as a means of genetically improving commercially important animals.

At the present time, we are exploring the use of transgenic technology to improve: a) freeze resistance using antifreeze protein genes, b) disease resistance using a lysozyme gene, and c) growth rates using a chimeric growth hormone gene. Since the research

using lysozyme is still at an early stage, we will confine our discussion to research involving antifreeze and growth hormone gene transfer. However, as will be seen from the following, the process required to successfully overcome regulatory, grower, and consumer acceptance hurdles will be the same regardless of the gene in question.

Antifreeze protein genes

Antifreeze proteins (AFP) are produced by a number of marine teleosts that inhabit waters at sub-zero (0 to -1.8°C) temperatures. These proteins are produced in two locations: 1) Liver, from whence they are secreted into the blood, resulting in plasma concentrations as high as 20 mg/ml, and reducing the freezing point of the fish extracellular fluids to safe levels, and 2) Epithelial tissues, where AFPs are produced to protect the tissues from damage due to direct ice contact at sub-zero temperatures (Fletcher et al. 2001). Many commercially important fish (salmon, trout, halibut, etc.) lack these proteins and their genes and, as a consequence, they will not survive if cultured in icy sea water (Hew et al. 1995; Fletcher et al. 1998).

In 1982, our transgenic studies were initiated by injecting winter flounder antifreeze genes into the fertilized eggs of Atlantic salmon. A full length gene encoding the major liver secretory AFP was used and the AFP transgene was successfully integrated into the salmon chromosomes, expressed, and found to exhibit Mendelian inheritance over 5 generations to date (Shears et al. 1991; Hew et al. 1999). Expressed levels of AFP in the blood of these fish are quite low (100 - 400 μg/ml) and is insufficient to confer any significant increase in freeze resistance to the salmon. However, the "proof of the concept" that salmon and other fish species can be rendered more freeze resistant by gene transfer has been established. The challenge now is to design an antifreeze gene construct(s) that will result in enhanced expression in appropriate tissues: epithelia and liver.

Sea cage production of Atlantic salmon in eastern Canada amounts to approximately $160 million (US) (~34,000 metric tonnes), and provides much-needed employment for over 3,000 people (CAIA 2003). However, the low water temperatures that prevail throughout much of this region during winter have restricted the salmon aquaculture industry to the most southerly part of the region. The successful production of a freeze resistant salmon broodstock could facilitate the expansion of aquaculture and economic development throughout the entire Atlantic coastal region. Although such a broodstock would likely be of value only to cold water regions, annual revenues in royalties for the use of this broodstock could be in the millions.

Growth hormone genes

All aquaculture ventures could benefit from the development of culture species with enhanced growth rates that would reduce the time required to raise fish (or shellfish) to market size. At present, it takes approximately 16-18 months of sea pen culture to

produce marketable Atlantic salmon in Atlantic Canada. If growth rates during this phase could be doubled, it may be possible to market the salmon following a single growing season and obviate the need for overwintering in sea-pens.

Growth hormone genes are normally expressed in the pituitary gland under the control of the central nervous system (CNS). In order to by-pass the CNS, it is necessary to modify the tissue specific elements of the gene so that expression can take place elsewhere. The AFP genes are expressed predominantly in the liver, and therefore we designed our gene construct using the ocean pout AFP promoter (opAFP) linked to the chinook salmon GH cDNA (Hew et al. 1995). Our GH gene transfer studies were initiated in 1989 with the injection of these constructs into fertilized salmon eggs.

The genomic integration frequency of the GH transgene was similar to that observed for the AFP genes (2-3 %). Less than half of these GH transgenics exhibited growth rates that were, on average, 3-6 times that of standard (control) salmon over a 30 month period. All of the GH-transgenic salmon that developed from the injected eggs were germ cell mosaics, and half of them failed to pass on the GH transgene to their offspring. Mendelian inheritance of the GH transgene and its rapid growth phenotype was established at the F_1 generation and has now been demonstrated through the second, third, fourth, and fifth generations.

In general, the transgenics grow most rapidly during their first year, slow to that of standard salmon at approximately one kilogram, and reach market size (3-4 kg) a year earlier than do non-transgenics grown commercially in Atlantic Canada.

In contrast to the regional value of freeze resistant salmon, the production of a broodstock with greatly enhanced growth rates could have world-wide application. World production of Atlantic salmon in 2001 was approximately one million metric tonnes, with an estimated value of $3,000 to $4,000 million US (Globefish 2003). Therefore, depending on the extent to which the industry applies this technology and on the royalty negotiated, annual revenues to the owners of the technology could exceed $50 million US.

Prospects and expectations for the future of transgenics

There is no doubt that transgenic techniques can be used develop fish for aquaculture with superior production characteristics. Such improvements could impart significant benefits to the ever increasing world population while also having a positive impact on marine fish stocks, and if applied judicially, on the marine environment itself. However, there are a number of factors to consider and weigh before the final product can enter the marketplace, and these can be grouped under the following headings:

- Basic science
- Biosafety (food safety; environmental protection; animal welfare)
- Consumer acceptance

Basic Science

Our lessons from salmon have taught us that:

1. Integration frequencies of injected transgenes into the Atlantic salmon genome will be low (2-3%).
2. The transgene can integrate into more than one chromosome, and more than one copy of the gene can integrate into a single chromosome.
3. The transgenes can be rearranged prior to integration, resulting in weak to no expression.
4. All of the founder generation fish will be somatic as well as germ cell mosaics, indicating that the transgene does not integrate into the chromosomes until as late as organogenesis.
5. A Mendelian inheritance pattern cannot be established until the third generation (F_2).
6. Transgenic fish homozygous for the transgene cannot be produced with certainty until the fourth generation (F_3).

Two general conclusions can be drawn from the above observations. The production of stable lines of desirable and commercially valuable broodstock is not a short term endeavour, particularly for species with a long breeding cycle, and the success of the final product is difficult to predict with certainty from the first two generations.

Biosafety

There are three areas to consider under this heading: a) assessment of the safety of the transgenic fish as food for human (or animal) consumption, b) assessment of the possible environmental impact of the living transgenic fish should they be introduced or escape into the wild, and c) health of the transgenic fish produced as a result of biotechnology.

Food safety issues will be dealt with by the relevant regulatory body, which is country specific, and also determined by the nature of the genetic modification. In order to bring transgenic Atlantic salmon to market in Canada or the U.S.A., the regulatory bodies involved (Health Canada and the FDA, respectively), will require data to demonstrate that the edible tissue is equivalent in composition to that of the product already on the market and that there is no change in allergenicity of the product. Fish do not possess genes that code for toxins. Thus, there can be no rational concern that insertion of the transgene into the host DNA could result in a toxic food product (Berkowitz and Kryspin-Sorensen 1994).

Environmental protection considerations are hard to deal with in general terms, since potential risks will depend on the species being cultivated, the area in which it is being cultivated, the nature of the transgenic modification, and the nature of the ecosystem into which transgenic individuals might possibly escape.

At present, salmon are cultured in cages that are located in coastal waters near to the shore. This brings with it a number of problems, one of which is the possibility that fish will escape and interact with the wild resource. When considering a transgenic salmon, it is essential that transgenic broodstock be maintained in secure, contained land-based facilities. Table fish, if they are to be cultured in cages, must be rendered sterile. To date, the only effective and publicly acceptable method of ensuring 100% sterility is the production of all-female triploids (Johnstone 1996; Devlin and Donaldson 1992)

Intensive cage culture of salmon in coastal waters can have a negative impact on the environment and on the natural wild stocks (Stewart 1997). The long-term effect of near-shore aquaculture on the sustainability of the coastal ecosystem is impossible to predict, particularly when growth in production is factored into the equation. Therefore, under certain circumstances, it may be preferable for aquaculture development to take place on land in high quality recirculating water systems, making aquaculture less dependent on good coastal water sites. The challenge of such land-based systems is their commercial viability; their advantage is that they offer growers greater control over disease, parasitic infections, feeding regimens, temperature, and photoperiod, making it possible to provide high-value fish in a sustainable, environmentally, and ecologically sound manner. Culture on land would also allow broodstock developers to fully domesticate farmed fish, and free them from concerns over changing the genetic make-up of domesticated fish from that of their wild relatives (Alestrøm 1995).

Animal welfare is an issue of concern to fish farmers, as well as animal rights groups and, indeed, to the public in general. Thus, the transgenic animal's overall general welfare and health must be of paramount importance throughout the life cycle of the fish. Whatever the transgenic modification, fish must be healthy and exhibit normal feeding and other behaviour patterns typical of domesticated species.

In Canada, the appropriate regulatory agencies for food safety and animal health are Health Canada (2004)(Silva and Buchanan 2000), and the Canadian Food Inspection Agency (CFIA). Environmental safety is regulated by Environment Canada (Canadian Environmental Protection Act (CEPA)) and the Department of Fisheries and Oceans. In the United States the appropriate agency is the Centre for Veterinary Medicine within the Food and Drug Administration. In the case of transgenic salmon, the transgenes and their products are considered as new animal drugs.

Consumer acceptance

It is important to think globally when considering consumer acceptance of transgenic technology. What may seem outlandish, unnatural, and unnecessary to inhabitants of one part of the planet may hold the key to increased prosperity, environmental remediation, and even survival elsewhere. It is also important to learn from past experience - no new technology is risk-free but the benefits may vastly outweigh the risks (for example, the

Green Revolution, and the development of prescription medicines).

In Europe, and to a lesser extent North America, increased risk aversion, distrust of Government and "Big Business", and a desire (in the absence of hunger) to return to nature has resulted in something approaching biotechnophobia. It will take time, and considerable dialogue between all those involved for this situation to change.

From the perspective of the public, information concerning genetically modified food must come from an objective, unbiased source. The consumer and the public should be kept informed about upcoming products in advance of their appearance in the marketplace. They must be certain that new products of biotechnology are safe, useful, and beneficial to their well being and to the environment. Producers must also be kept informed about new products, and given the confidence that they will not lose their markets because they choose to grow fish using the most advanced methods available to them.

At all costs we, as citizens, must avoid negativism and help the public to recognise that transgenic technologies are a positive symbol of modern scientific thinking (Gillard 2003).

Summary

Over the past 20 years we have generated stable lines of transgenic Atlantic salmon possessing either antifreeze protein (AFP) or chimeric salmon growth hormone (GH) genes.

Our transgenic studies were initiated in 1982 with the goal of producing salmon with enhanced cold and freeze resistance for culture in coastal waters along the east coast of Canada. A full length gene encoding one of the major AFPs that is secreted by the winter flounder liver was injected into fertilized Atlantic salmon eggs. The AFP transgene was successfully integrated into the salmon genome, expressed, and found to exhibit Mendelian inheritance over multiple generations. Expressed levels of AFP in the blood of these fish are insufficient to confer any significant increase in freeze resistance on the salmon. However, proof of the concept that salmon and other fish species can be rendered more freeze resistant by gene transfer has been established. The challenge now is to design an antifreeze gene construct(s) that will result in enhanced expression in appropriate tissues.

Our GH gene transfer studies were initiated in 1989 using the ocean pout antifreeze gene promoter (opAFP) linked to the chinook salmon GH cDNA. In one line of GH transgenic salmon the data indicate that a single copy of the GH transgene was integrated into the chromosomal DNA. Mendelian inheritance of the transgene and its rapid growth phenotype has now been demonstrated over five generations. Laboratory studies indicate that our GH transgene enhances growth rates to the point where Atlantic salmon can

reach market size (4-6 kg) a year earlier than can non-transgenics grown commercially in Atlantic Canada.

Sea cage production of Atlantic salmon in eastern Canada amounts to approximately $160 million (US). However, the low water temperatures that prevail throughout much of this region during winter have restricted the salmon aquaculture industry to the most southerly part of the region. The successful production of a freeze resistant salmon broodstock could facilitate the expansion of aquaculture throughout the entire coastal region of Atlantic Canada. Although such a broodstock would likely be of value only to cold water regions, annual revenues in royalties for the use of this broodstock could be in the millions.

In contrast to the regional value of freeze resistant salmon, the production of a broodstock with greatly enhanced growth rates could have world-wide application. World production of Atlantic salmon in 2001 was approximately a million metric tonnes, with an estimated value of $3,000 to $4,000 million US. Therefore depending on the extent to which the industry applies this technology and on the royalty negotiated, annual revenues to the owners of the technology could exceed $50 million US.

The GH gene transfer technology was patented by Memorial University and the Toronto Hospital for Sick Children, licensed to Aqua Bounty Technologies, Inc. and is currently under review, or being prepared for, by various government regulatory authorities in United States and Canada for use in commercial aquaculture ventures. Our experience with the regulatory authorities, the industry, and the press indicates that the successful introduction of transgenics into the aquaculture industry involves not only basic science but also food safety, environmental safety, animal welfare, producer acceptance, and consumer acceptance. This brief communication centers on our experience with Atlantic salmon and outlines our plans and progress towards demonstrating that transgenic fish are safe for both the consumer and the environment.

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Participant Discussions

Donaldson: You mentioned the need to grow transgenic fish in land based containment. There is one company using facilities on land, specifically with nontransgenic Atlantic and Pacific salmon in concrete tanks (eco friendly facility in Vancouver).

Muir: Was the gene circular when injected?

Fletcher: No, we linearized it.

Muir: Was it cut out, spliced somehow into the genome?

Fletcher: The GH gene construct was reorganized when integrated into the salmon genome. However, its function remained intact resulting in rapid growth.

Kapuscinski: When you say multiple repeat, do you mean microsatellite?

Fletcher: No, what I mean is that the transgene integrated into a multiple repeat region of the chromosomal DNA.

Kapuscinski: Was the actual (growth hormone) protein size the same, and was there any variation between transgenic and wild type?

Fletcher: It doesn't seem likely that the actual hormone changed. The promoter is still functional, and the transgene appears to be fully functional.

Masri: We performed a western blot and the gene product is about the same size as its original construct from salmon. I think probably you have the same size in gene constructs.

Unknown: It is the same size as native growth hormone. King salmon and Atlantic salmon are 98% similar.

Poon: One of the mysteries in our lab, working on fishes, is detecting the growth hormone mRNA in the muscle. How can fish grow so fast without any hormone being detected? Is it because of the half life of the hormone? The binding protein?

Fletcher: Haven't got a clue. More work for us scientists.

Bughio: Were the vector sequences removed from the final construct that was microinjected into the embryos that developed into transgene fish?

Fletcher: In our case no, the transgene was linearized prior to microinjection.

Bughio: Bob Devlin and group have published data on deformities in the transgenic fish. Have you observed any deformities or health and welfare problems in transgenic Atlantic salmon?

Fletcher: Yes, sometimes as many as 5-25% show morphological irregularities. However, selective breeding for rapid growth also results in similar irregularities. In general, the salmon with irregularities are healthy, eat well, and grow rapidly.

Bughio: Are the transgenic fish to be commercialized sterile?

Fletcher: Yes, that is the plan. There are two basic reasons for doing this. In the first instance, sterile fish will not be capable of reproduction and thus would be incapable of interbreeding with wild salmon. The second reason is to protect the company's intellectual property. Currently the industry cultures salmon that are fully capable of reproduction. Therefore the question that needs to be resolved is whether sterile or 99.8% sterile transgenic salmon are a greater risk to the environment than fertile non-transgenics.

Bughio: Which techniques will be used to verify/confirm the induction of triploidy/sterility of the transgenic Atlantic salmon?

Fletcher: Triploidy is easily confirmed (Tillman Benfey's session will discuss this more tomorrow).

Bughio: What is the estimated number of transgenic eggs available at your research and development facility in Canada, in 2004??

Fletcher: Most females produce about 5,000 to 10,000 thousand eggs at a time. Therefore the number of eggs that we would have at our facility during spawning season would depend on the number of mating crosses that we carry out. I can't give an exact number. However, it certainly could exceed a half a million. One to two million transgenic Atlantic salmon eggs will be available in 2004.

Donaldson: Did anyone look at the lipid levels of in the liver?

Fletcher: No, since we don't eat the liver, no one has measured lipid levels.

EUROPEAN PERSPECTIVE ON GM FISH USE AND REGULATION

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Abstract

Now that fish of many species have been subjected to the transgenic technology, and some species such as tilapia (*Oreochromis niloticus*), Atlantic Salmon (*Salmo salar*), and mud loach (*Misgurnus microlepis*) are effectively growth enhanced, there has been a sudden proliferation of regulations covering the possible commercial exploitation and release of such fish. Regulations and conditions applicable to European countries are specifically considered in this contribution. Parameters of particular relevance include the characteristics of the fish, containment, possible release into the aquatic environment, characteristics of all the genetic elements included in the transgene constructed, and labeling of any commercial food products produced from such fish.

Résumé

Maintenant que des poissons de nombreuses espèces ont été utilisés aux fins de transgénèse et que la croissance d'espèces comme le tilapia (*Oreochromis niloticus*), le saumon atlantique (*Salmo salar*) et la loche (*Misgurnus mizolepis*) a été améliorée avec succès, il y a eu une prolifération soudaine de règlements relatifs à l'exploitation commerciale et à la libération possibles de tels poissons. Dans le présent document, nous prêtons une attention particulière aux règlements et aux conditions applicables à cet égard en Europe. Les paramètres particulièrement pertinents comprennent les caractéristiques des poissons, le confinement, la libération possible dans le milieu aquatique, les caractéristiques de tous les éléments génétiques compris dans le transgène produit et l'étiquetage de tout produit alimentaire commercial préparé à partir de tels poissons.

Brief history of the technology

Over the past 20 years a number of laboratories around the world have helped develop the technology by which transgenic fish are produced. This has most frequently involved microinjection of fertilized eggs with linear DNA sequences, followed by subsequent recovery of fish with integrated transgenes and establishment of breeding lines. Fish of many species have been made transgenic, including model fish species such as zebrafish, medaka, and goldfish, and commercially significant species such as rainbow trout, chinook, coho, and Atlantic salmon, tilapia, common carp, mud loach, and European and channel catfish.

The parameter most frequently modified by the fish GM technology is growth rate, via the expression of extra copies of growth hormone genes driven by a range of promoters. In addition, other characteristics such as freeze resistance, cold tolerance, metabolism, sterility, and disease resistance have also been targeted.

The terminology

The terms transgenic and genetically manipulated/genetically modified (GM) are used interchangeably, and the resulting fish are referred to as GMOs (genetically modified organisms) or LMOs (living modified organisms). None of this terminology is absolutely ideal in that fish produced by gynogenesis or sex reversal could be equally well described as being modified as a result of gene manipulation. Some authorities seek to circumvent this confusion by defining GM fish as resulting from the alteration of the genetic material in a non-natural way, i.e., not by making by natural recombination (see Annex 1A of the Environment Canada regulation).

The introduced DNA sequence is often referred to as a DNA construct and this usually includes a regulatory sequence (often loosely styled 'the promoter'), and the chosen coding sequence or gene. This latter sequence can be a cDNA (without introns) or genomic (with introns).

When the resulting GM fish are being maintained outside the laboratory, it is customary to ensure their complete containment. This can be physical as in a land locked lake or by maintenance in warm water that is surrounded by cold water, or biological as by sterility.

The GM fish are usually described as being hemizygous with respect to the transgene, meaning that only a single chromosome in the diploid chromosome set carries an integrated transgene copy. If the gene construct is entirely of fish origin, then it is often described as being an all-fish construct. If the construct is made up of sequences derived entirely from the species subjected to transgenesis, then the resulting fish are described as autotransgenic.

The European perspective

In this paper I will outline the European attitudes and regulations, as well as the UK ones. Each European country may well have its own regulations, but is also bound by Environment Canada regulations if imports are proposed for Canada. While each European country is bound to implement the Environment Canada regulations, they may interpret them slightly differently.

In terms of Europe the documents to be aware of are (a) the Cartagena Protocol on Biosafety, signed in January 2000 following the Rio summit and declaration on

Environmental Issues and Development, and (b) the directive from the European Parliament on deliberate GMO release, dated March 2001.
In terms of the UK, the documents are as follows:

a) The April 1994 issue by the Department of the Environment entitled "Genetic modification of fish - a UK perspective".
b) Guidance Notes published by the Department of the Environment, Transport and the Regions (DETR/ACRE Guidance Notes 12) in November 1999.
c) The Report by the Royal Society UK in May 2001 entitled "The Use of Genetically Modified Animals".
d) The Advice of the Advisory Committee on Release to the Environment (ACRE Publication) in July 2001.
e) The Statutory Instrument 2002 No. 2443 issued by the UK Department for Environment, Food and Rural Affairs (DEFRA) on Environmental Protection, entitled Genetically Modified Organisms (Deliberate Release) which came into force in October 2002.
f) Note also that in the UK, all transgenic animals are categorized as 'harmful mutants' under the terms of the Home Office regulations on Animal Experiments. The scientist doing the work requires a separate Home Office License for each group of experiments, and the facilities themselves have to be licensed and are regularly inspected to ensure the good welfare of the animals.

Noteworthy points extracted from the documentation

a) Royal Society Report

This report picks out future developments with GM fish for special mention. "An environmental concern is the escape of GM fish and their breeding with the natural population. Phenotype changes due to the genetic modifications may provide the GM animals with a competitive advantage over their wild relatives for food, shelter, mates, and suitable breeding sites."

And again "Despite the potential for sterilizing GM fish, the Royal Society of Canada, in its recent report on biotechnology and food, concluded that the consequences of genetic and ecological interaction between GM and wild fish were uncertain as was the utility of attempting to render GM fish sterile. In particular, the Royal Society of Canada recommended a moratorium on rearing GM fish in aquatic net-pens, with approval for commercial production being conditional on rearing of the fish in land locked facilities. The Royal Society endorses this recommendation."

b) Definition of what is and what is not GM in the Environment Canada documentation

What is included — (i) recombinant DNA technology, (ii) technology involving direct introduction into an organism of heritable material prepared outside the organism, and (iii) cell fusion (including protoplast fusion) or cell hybridization where live cells with

new combinations of genetic material are formed, by means of methods that do not occur naturally.

What is not included — (i) in vitro fertilization, (ii) natural processes such as conjugation, transduction and transformation, and (iii) polyploidy induction.

Potential adverse effects of GMOs listed in European legislation

a) Disease to humans including allergic or toxic effects
b) Disease to animals and plants
c) Effects on dynamics of populations
d) Altered susceptibility to pathogens
e) Compromising therapeutic treatments to humans, animals, and plants
f) Effects on carbon or nitrogen recycling

Evaluation of potential consequences of adverse effects of GMOs listed in European legislation

a) Gene construct and inclusion of viral sequences or potentially toxic sequences
b) Number of gene copies in GMO
c) Any genetic position effects
d) Number of GMOs to be released
e) The environment into which release will occur
f) Control measures during release, i.e., detailed risk management

EC rules for member states on acceptance

a) "When product containing a GMO, as or in products, is placed on the market, and where such a product has been properly authorized under this Directive, a Member State may not prohibit, restrict or impede the placing on the market of the GMOs as or in products...."

b) Labeling — All GMOs and GMO products must be clearly labeled as such. It is worth emphasizing that there is an increasing awareness within UK and EU that with respect to both labeling and testing of GMOs for adverse effects, present regulations for GMOs go far beyond those currently in place for non GMOs.

Levels of risk with GM fish implicit in DEFRA regulations in UK

Minimal — tilapia
Low — rainbow trout
Median — carp and salmon

The European Parliament and the Council of the European Union

- Having regard to the Treaty establishing the European Community, and in particular Article 95.
- Having regard to the proposal from the Commission.
- Having regard to the opinion of the Economic and Social Committee.

- Acting in accordance with the procedure laid down in Article 251 of the Treaty, in the light of the joint text approved by the Conciliation Committee on 20 December 2000.

(1) The Report of the Commission on the Review of Council Directive 90/220/EEC of 23 April 1990 on the deliberate release into the environment of genetically modified organisms, adopted on 10 December 1996, and identified a number of areas where improvement is needed.

(2) There is a need for clarification of the scope of Directive 90/220/EEC and of the definitions therein.

(3) Directive 90/220/EEC has been amended. Now that new amendments are being made to the Directive, it is desirable, for reasons of clarity and rationalization, that the provisions in question should be recast.

(4) Living organisms, whether released into the environment in large or small amounts for experimental purposes or as commercial products, may reproduce in the environment and cross national frontiers thereby (03 C 139, 4.5.1998, p. 1). The effects of such releases on the environment may be irreversible.

(5) The protection of human health and the environment requires that due attention be given to controlling risks from the deliberate release into the environment of genetically modified organisms (GMOs).

(6) Under the Treaty, action by the Community relating to the environment should be based on the principle that preventive action should be taken.

(7) It is necessary to approximate the laws of the Member States concerning the deliberate release into the environment of GMOs and to ensure the safe development of industrial products utilizing GMOs.

(8) The precautionary principle has been taken into account in the drafting of this Directive and must be taken into account when implementing it.

(9) Respect for ethical principles recognized in a Member State is particularly important. Member States may take into consideration ethical aspects when GMOs are deliberately released or placed on the market as or in products.

(10) For a comprehensive and transparent legislative framework, it is necessary to ensure that the public is consulted by either the Commission or the Member States during the preparation of measures and that they are informed of the measures taken during the implementation of this Directive.

(11) Placing on the market also covers import. Products containing and/or consisting of GMOs covered by this Directive cannot be imported into the Community if they do not comply with its provisions.

(12) Making GMOs available to be imported or handled in bulk quantities, such as agricultural commodities, should be regarded as placing on the market for the purpose of this Directive.

(13) The content of this Directive duly takes into account international experience in this field.

(Acts whose publication is obligatory)
DIRECTIVE 2001/18/EC OF The European PARLIAMENT and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90I2201EEC.

Trade commitments and should respect the requirements of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. As soon as possible, and in any case before July 2001, the Commission should, in the context of the ratification of the Protocol, submit the appropriate proposals for its implementation.

(14) Guidance on the implementation of provisions related to the definition of the placing on the market in this Directive should be provided by the Regulatory Committee.
(15) When defining 'genetically modified organisms' for the purpose of this Directive, human beings should not be considered as organisms.
(16) The provisions of this Directive should be without prejudice to national legislation in the field of environmental liability, while Community legislation in this field needs to be complemented by rules covering liability for different types of environmental damage in all areas of the European Union. To this end the Commission has undertaken to bring forward a legislative proposal on environmental liability before the end of 2001, which will also cover damage from GMOs.
(17) This Directive should not apply to organisms obtained through certain techniques of genetic modification which have conventionally been used in a number of applications and have a long safety record.
(18) It is necessary to establish harmonized procedures and criteria for the case-by-case evaluation of the potential risks arising from the deliberate release of GMOs into the environment.
(19) A case-by-case environmental risk assessment should always be carried out prior to a release. It should also take due account of potential cumulative long-term effects associated with the interaction with other GMOs and the environment.
(20) It is necessary to establish a common methodology to carry out the environmental risk assessment based on independent scientific advice. It is also necessary to establish common objectives for the monitoring of GMOs after their deliberate release or placing on the market as or in products. Monitoring of potential cumulative long-term effects should be considered as a compulsory part of the monitoring plan.
(21) Member States and the Commission should ensure that systematic and independent research on the potential risks involved in the deliberate release or the placing on the market of GMOs is conducted. The necessary resources should be secured for such research by Member States and the Community in accordance with their

budgetary procedures and independent researchers should be given access to all relevant material, while respecting intellectual property rights.

(22) The issue of antibiotic-resistance genes should be taken into particular consideration when conducting the risk assessment of GMOs containing such genes.

(23) The deliberate release of GMOs at the research stage is in most cases a necessary step in the development of new products derived from, or containing GMOs.

(24) The introduction of GMOs into the environment should be carried out according to the 'step by step' principle. This means that the containment of GMOs is reduced and the scale of release increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken.

(25) No GMOs, as or in products, intended for deliberate release are to be considered for placing on the market without first having been subjected to satisfactory field testing at the research and development stage in ecosystems which could be affected by their use.

(26) The implementation of this Directive should be carried out in close liaison with the implementation of other relevant instruments such as Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. In this context the competent authorities concerned with the implementation of this Directive and of those instruments, within the Commission and at national level, should coordinate their action as far as possible.

(27) Concerning the environmental risk assessment for part C, risk management, labeling, monitoring, information to the public and safeguard clause, this Directive should be a point of reference for GMOs as or in products authorized by other Community legislation which should therefore provide for a specific environmental risk assessment, to be carried out in accordance with the principles set out in Annex II and on the basis of information specified in Annex III without prejudice to additional requirements laid down by the Community legislation mentioned above, and for requirements as regards risk management, labeling, monitoring as appropriate, information to the public and safeguard clause at least equivalent to that laid down in this Directive. To this end it is necessary to provide for cooperation with the Community and Member State bodies mentioned in this Directive for the purpose of its implementation.

Directive as last amended by Commission Directive

Principles for the Environmental Risk Assessment

This Annex describes in general terms the objective to be achieved, the elements to be considered and the general principles and methodology to be followed to perform the environmental risk assessment (e.r.a.) referred to in Articles 4 and 13. It will be supplemented by guidance notes to be developed in accordance with the procedure laid down in Article 30(2). These guidance notes shall be completed by 17 October 2002.

With a view to contributing to a common understanding of the terms ‘direct, indirect, immediate and delayed’ when implementing this Annex, without prejudice to further guidance in this respect and in particular as regards the extent to which indirect effects can and should be taken into account, these terms are described as follows:

— ‘Direct effects’ refers to primary effects on human health or the environment which is a result of the GMO itself and which do not occur through a causal chain of events;
— ‘Indirect effects’ refers to effects on human health or the environment occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management.

Observations of indirect effects are likely to be delayed:

— ‘immediate effects’ refers to effects on human health or the environment which are observed during the period of the release of the GMO. Immediate effects may be direct or indirect;
— ‘delayed effects’ refers to effects on human health or the environment which may not be observed during the period of the release of the GMO, but become apparent as a direct or indirect effect either at a later stage or after termination of the release.

A general principle for environmental risk assessment is also that an analysis of the ‘cumulative long-term effects’ relevant to the release and the placing on the market is to be carried out. ‘Cumulative long-term effects’ refers to the accumulated effects of consents on human health and the environment, including inter alia flora and fauna, soil fertility, soil degradation of organic material, the feed/ food chain, biological diversity, animal health and resistance problems in relation to antibiotics.

A. Objective

The objective of an e.r.a. is, on a case by case basis, to identify and evaluate potential adverse effects of the GMO, direct and indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have. The e.r.a. should be conducted with a view to identifying if there is a need for risk management and if so, the most appropriate methods to be used.

B. General Principles

In accordance with the precautionary principle, the following general principles should be followed when performing the e.r.a.:
— Identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations;

— The e.r.a. should be carried out in a scientifically sound and transparent manner based on available scientific and technical data;
— The e.r.a. should be carried out on a case by case basis, meaning that the required information may vary depending on the type of the GMOs concerned, their intended use and the potential receiving environment, taking into account, i.e., GMOs already in the environment;
— If new information on the GMO and its effects on human health or the environment becomes available, the e.r.a. may need to be readdressed in order to:
    — determine whether the risk has changed;
    — determine whether there is a need for amending the risk management accordingly.

C. Methodology

Cl. Characteristics of GMOs and releases

Depending on the case the e.r.a. has to take into account the relevant technical and scientific details regarding characteristics of:
— The recipient or parental organism(s);
— The genetic modification(s), be it inclusion or deletion of genetic material, and relevant information on the vector and the donor;
— The GMO;
— The intended release or use including its scale;
— The potential receiving environment; and
— The interaction between these.
Information from releases of similar organisms and organisms with similar traits and their interaction with similar environments can assist the e.r.a.

C.2. Steps in the era

In drawing conclusions for the e.r.a. referred to in Articles 4, 6, 7 and 13 the following points should be addressed:

1. Identification of characteristics which may cause adverse effects:

Any characteristics of the GMOs linked to the genetic modification that may result in adverse effects on human health or the environment shall be identified. A comparison of the characteristics of the GMO(s) with those of the non-modified organism under corresponding conditions of the release or use will assist in identifying the particular potential adverse effects arising from the genetic modification. It is important not to discount any potential adverse effect on the basis that it is unlikely to occur.

Potential adverse effects of GMOs will vary from case to case, and may include:
— Disease to humans including allergenic or toxic effects (see for example items ll.A.1 1. and lI.C.2(i) in Annex Ill A, and B 7 in Annex Ill B);

— Disease to animals and plants including toxic, and where appropriate, allergenic effects (see for example items ILA.11. and l1.C.2(i) in Annex Ill A, and B 7 and D 8 in Annex ill B);
— Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations (see for example items IV B 8, 9 and 12 in Annex 111 A);
— Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors;
— Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments, for example by transfer of genes conferring resistance to antibiotics used in human or veterinary medicine (see for example items 1l.A.1 1(e) and ILC.2(i)(iv) in Annex ill A);
— Effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material (see for example items ILA.1 1(1) and IV.B.1 5 in Annex 111 A, and D 11 in Annex ill B).

1. Adverse effects may occur directly or indirectly through mechanisms which may include:

— The spread of the GMO(s) in the environment, the transfer of the inserted genetic material to other organisms, or the same organism whether genetically modified or not,
— Phenotypic and genetic instability,
— Interactions with other organisms,
—Changes in management, including, where applicable, in agricultural practices.

2. Evaluation of the potential consequences of each adverse effect, if it occurs

The magnitude of the consequences of each potential adverse effect should be evaluated.

This evaluation should assume that such an adverse effect will occur. The magnitude of the consequences is likely to be influenced by the environment into which the GMO(s) is (are) intended to be released and the manner of the release.

3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

A major factor in evaluating the likelihood or probability of adverse effects occurring is the characteristics of the environment into which the GMO(s) is intended to be released, and the manner of the release.

4. Estimation of the risk posed by each identified characteristic of the GMO(s)

An estimation of the risk to human health or the environment posed by each identified characteristic of the GMO which has the potential to cause adverse effects should be made as far as possible,

given the state of the art, by combining the likelihood of the adverse effect occurring and the magnitude of the consequences, if it occurs.

5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)

The risk assessment may identify risks that require management and how best to manage them, and a risk management strategy should be defined.

6. Determination of the overall risk of the GMO(s)

An evaluation of the overall risk of the GMO(s) should be made taking into account any risk management strategies which are proposed.

D. Conclusions on the potential environmental impact from the release or the placing on the market of GMOs

On the basis of an e.r.a. carried out in accordance with the principles and methodology outlined in sections B and C, information on the points listed in sections D or D2 should be included, as appropriate, in notifications with a view to assisting in drawing conclusions on the potential environmental impact from the release or the placing on the market of GMOs:

D.1. In the case of GMOs other than higher plants

1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).
2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s).
3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.
4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable).
5. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.
6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).
7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.

8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-CMOs.

D.2. In the case of genetically modified higher plants (GMHP)

1. Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.
2. Any selective advantage or disadvantage conferred to the GMHP.
3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.
4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable).
5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable) parasites and pathogens.
6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s).
7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.
8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).
9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.

Information Required in Notifications Concerning Release of Genetically Modified Organisms other than Higher Plants

I. GENERAL INFORMATION

A. Name and address of the notifier (company or institute)

B. Name, qualifications and experience of the responsible scientist(s)

C. Title of the project

II. INFORMATION RELATING TO THE GMO

A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s):

1. Scientific name,
2. Taxonomy,
3. Other names (usual name, strain name, etc.),
4. Phenotypic and genetic markers,
5. Degree of relatedness between donor and recipient or between parental organisms,
6. Description of identification and detection techniques,
7. Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques,
8. Description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts,
9. Organisms with which transfer of genetic material is known to occur under natural conditions,
10. Verification of the genetic stability of the organisms and factors affecting it,
11. Pathological, ecological and physiological traits:
    (a) Classification of hazard according to existing Community rules concerning the protection of human health and/or the environment;
    (b) Generation time in natural ecosystems, sexual and asexual reproductive cycle;
    (c) Information on survival, including seasonability and the ability to form survival structures;
    (d) Pathogenicity infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonize other organisms;
    (e) Antibiotic resistance and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;
    (f) Involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.
12. Nature of indigenous vectors:

(a) Sequence;
(b) Frequency of mobilization;
(c) Specificity
(d) Presence of genes which confer resistance.

13. History of previous genetic modifications.

B. Characteristics of the vector

1. Nature and source of the vector,
2. Sequence of transposons, vectors and other non-coding genetic segments used to construct the GMO and to make the introduced vector and insert function in the GMO,
3. Frequency of mobilization of inserted vector and/or genetic transfer capabilities and methods of determination,
4. Information on the degree to which the vector is limited to the DNA required to perform the intended function.

C. Characteristics of the modified organism

1. Information relating to the genetic modification:
   (a) Methods used for the modification;
   (b) Methods used to construct and introduce the insert(s) into the recipient or to delete a sequence;
   (c) Description of the insert and/or vector construction;
   (d) Purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function;
   (e) Methods and criteria used for selection;
   (f) Sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in. question with particular reference to any known harmful sequence.
2. Information on the final GMO:
   (a) Description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;
   (b) Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;
   (c) Stability of the organism in terms of genetic traits;
   (d) Rate and level of expression of the new genetic material. Method and sensitivity of measurement;
   (e) Activity of the expressed protein(s);
   (f) Description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector
   (g) Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;
   (h) History of previous releases or uses of the GMO;

(i) Considerations for human health and animal health, as well as plant health:

(i) Toxic or allergenic effects of the GMOs and/or their metabolic products;

(ii) Comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity

(iii) Capacity for colonization;

(iv) If the organism is pathogenic to humans who are immunocompetent:

— Diseases caused and mechanism of pathogenicity including invasiveness and virulence,

— Communicability,

— Infective dose,

— Host range, possibility of alteration,

— Possibility of survival outside of human host,

— Presence of vectors or means of dissemination,

— Biological stability,

— Antibiotic resistance patterns,

— Allergenicity,

— Availability of appropriate therapies.

(v) Other product hazards.

III. Information Relating to the Conditions of Release and the Receiving Environment

A. Information on the release

1. Description of the proposed deliberate release, including the purpose(s) and foreseen products,
2. Foreseen dates of the release and time planning of the experiment including frequency and duration of releases,
3. Preparation of the site previous to the release,
4. Size of the site,
5. Method to be used for the release,
6. Quantities of GMOs to be released,
7. Disturbance on the site (type and method of cultivation, mining, irrigation, or other activities),
8. Worker protection measures taken during the release,
9. Post-release treatment of the site,
10. Techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment,
11. Information on, and results of, previous releases of the GMOs, especially at different scales and in different ecosystems.

B. Information on the environment (both on the site and in the wider environment):

1. Geographical location and grid reference of the site(s) (in case of notifications under part C the site(s) of release will be the foreseen areas of use of the product),
2. Physical or biological proximity to humans and other significant biota,
3. Proximity to significant biotopes, protected areas, or drinking water supplies,
4. Climatic characteristics of the region(s) likely to be affected,
5. Geographical, geological and pedological characteristics,
6. Flora and fauna, including crops, livestock and migratory species,
7. Description of target and non-target ecosystems likely to be affected,
8. A comparison of the natural habitat of the recipient organism with the proposed site(s) of release,
9. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

IV. Information Relating to the Interactions between the CMOs and the Environment

A. Characteristics affecting survival, multiplication and dissemination

1. Biological features which affect survival, multiplication and dispersal,
2. Known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.),
3. Sensitivity to specific agents.

B. Interactions with the environment

1. Predicted habitat of the GMOs, JT fLUC\ U IC1JS P0
2. Studies of the behavior and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses,
3. Genetic transfer capability
   (a) Postrelease transfer of genetic material from GMOs into organisms in affected ecosystems;
   (b) post release transfer of genetic material from indigenous organisms to the GMOs;
4. Likelihood of post release selection leading to the expression of unexpected and/or undesirable traits in the modified organism, measures employed to ensure and to venly genetic stability.
5. Description of genetic traits which may prevent or minimize dispersal of genetic material.
6. Methods to verify genetic stability, routes of biological dispersal, known or potential modes of interaction with the disseminating agent,

   — including inhalation, ingestion, surface contact, burrowing, etc.,

7. Description of ecosystems to which the GMOs could be disseminated,
8. Potential for excessive population increase in the environment,
9. Competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s),
10. Identification and description of the target organisms if applicable,
    —anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable,
11. Identification and description of non-target organisms which may be adversely affected by the release of the GMO and the anticipated mechanisms of any identified adverse interaction,
12. Likelihood of post release shifts in biological interactions or in host range,
13. Known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens,
14. Known or predicted involvement in biogeochemical processes,
15. Other potential interactions with the environment.

V. Information on Monitoring, Control, Waste Treatment and Emergency Response Plans

A. Monitoring techniques

1. Methods for tracing the GMOs, and for monitoring their effects,
2. Specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques,
3. Techniques for detecting transfer of the donated genetic material to other organisms,
4. Duration and frequency of the monitoring.

B. Control of the release

1. Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of release or the designated area for use,
2. Methods and procedures to protect the site from intrusion by unauthorized individuals,
3. Methods and procedures to prevent other organisms from entering the Site.

C. Waste treatment

1. Type of waste generated,
2. Expected amount of waste,
3. Description of treatment envisaged.

D. Emergency response plans

1. Methods and procedures for controlling the GMOs in case of unexpected spread,

2. 2. Methods for decontamination of the areas affected, for example eradication of the GMOs,
3. Methods for disposal or sanitation of plants, animals, soils, etc., that were exposed during or after the spread,
4. Methods for the isolation of the area affected by the spread,
5. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

Additional Information

This Annex describes in general terms the additional information to be provided in the case of notification for placing on the market and information for labeling requirements regarding GMOs as or in product to be placed on the market, and GMO exempted under Article 2(4), second subparagraph. It will be supplemented by guidance notes, as regards the description of how the product is intended to be used, to be developed in accordance with the procedure laid down in Article 30(2). The labeling of exempted organisms as required by Article 26 shall be met by providing appropriate recommendations for, and restrictions on, use:

A. The following information shall be provided in the notification for placing on the market of GMOs as or in product in addition to that of Annex III:
    1. Proposed commercial names of the products and names of GMOs contained therein, and any specific identification, name or code used by the notifier to identify the GMO. After the consent any new commercial names should be provided to the competent authority,
    2. Name and full address of the person established in the Community who is responsible for the placing on the market, whether it is the manufacturer, the importer or the distributor,
    3. Name and full address of the supplier(s) of control samples,
    4. Description of how the product and the GMO as or in product are intended to be used. Differences in use or management of the GMO compared to similar non-genetically modified products should be highlighted,
    5. Description of the geographical area(s) and types of environment where the product is intended to be used within the Community, including, where possible, estimated scale of use in each area,
    6. Intended categories of users of the product, e.g., industry, agriculture, and skilled trades, consumer use by public at large,
    7. Information on the genetic modification for the purposes of placing on one or several registers modifications in organisms, which can be used for the detection and identification of particular GMO products to facilitate post-marketing control and inspection. This information should include where appropriate the lodging of samples of the GMO or its genetic material, with the competent authority and details of nucleotide sequences or other type of information which is necessary to identify the GMO product and its

progeny, for example the methodology for detecting and identifying the GMO product, including experimental data demonstrating the specificity of the methodology. Information that cannot be placed, for confidentiality reasons, in the publicly accessible part of the register should be identified,

8. Proposed labeling on a label or in an accompanying document. This must include, at least in summarized form, a commercial name of the product, a statement that 'This product contains genetically modified organisms', the name of the GMO and the information referred to in point 2. The labeling should indicate how to access the information in the publicly accessible part of the register.

B. The following information shall be provided in the notification, when relevant, in addition to that of point A, in accordance with Article 13 of this Directive:

1. Measures to take in case of unintended release or misuse,
2. Specific instructions or recommendations for storage and handling,
3. Specific instructions for carrying out monitoring and reporting to the notifier and, if required, the competent authority, so that the competent authorities can be effectively informed of any adverse effect. These instructions should be consistent with Annex V part C,
4. proposed restrictions in the approved use of the GMO, for example where the product may be used and for what purposes,

Monitoring Plan

This Annex describes in general terms the objective to be achieved and the general principles to be followed to design the monitoring plan referred to in Articles 13(2), 19(3) and 20. It will be supplemented by guidance notes to be developed in accordance with the procedure laid down in Article 30(2).

These guidance notes shall be completed by 17 October 2002.

A. Objective

The objective of a monitoring plan is to:

— confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the e.r.a. are correct, and

— identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated.

B. General principles

Monitoring, as referred to in Articles 1 3, 19 and 20, takes place after the consent to the placing of a GMO on the market.

The interpretation of the data collected by monitoring should be considered in the light of other existing environmental conditions and activities. Where changes in the environment are observed, further assessment should be considered to establish whether they are a consequence of the GMO or its use, as such changes

may be the result of environmental factors other than the placing of the GMO on the market.
Experience and data gained through the monitoring of experimental releases of GMOs may assist in designing the post marketing monitoring regime required for the placing on the market of GMOs as or in products.

C. Design of the monitoring plan
The design of the monitoring plan should:

1. Detailed on a case by case basis taking into account the e.r.a.
2. Take into account the characteristics of the GMO, the characteristics and scale of its intended use and the range of relevant environmental conditions where the GMO is expected to be released,
3. Incorporate general surveillance for unanticipated adverse effects and, if necessary, (case-) specific monitoring focusing on adverse effects identified in the e.r.a.:
    3.1. whereas case-specific monitoring should be carried out for a sufficient time period to detect immediate and direct as well as, where appropriate, delayed or indirect effects which have been identified in the e.r.a.,
    3.2. Whereas surveillance could, if appropriate, make use of already established routine surveillance practices such as the monitoring of agricultural cultivars, plant protection, or veterinary and medical products. An explanation as to how relevant information collected through established routine surveillance practices will be made available to the consent-holder should be provided.
4. Facilitate the observation, in a systematic manner, of the release of a GMO in the receiving environment and the interpretation of these observations with respect to safety to human health or the environment.
5. identify who (notifier, users) will carry out the various tasks the monitoring plan requires and who is responsible for ensuring that the monitoring plan is set into place and carried out appropriately, and ensure that there is a route by which the consent holder and the competent authority will be informed on any observed adverse effects on human health and the environment. (Time points and intervals for reports on the results of the monitoring shall be indicated).
6. give consideration to the mechanisms for identification and confirming any observed adverse effects on human health and environment and enable the consent holder or the competent authority, where appropriate, to take the measures necessary to protect human health and the environment.

References

ACRE: Advisory Committee on Release to the Environment
www.defra.gov.uk/environment/acre/pubs.htm

Adoption of the Cartagena Protocols on Biosafety. (2000) (CPB; www.biodiv.com)

DEFRA Statutory Instrument on Genetically Modified Organisms (Deliberate Release) www.opsi.gov.uk/si/si2002/20022443.htm.

Directive 2001/18/EC of the European Parliament of 12/3/01 on the deliberate release into the environment of genetically modified organisms. http://europa.eu.int/scadplus/leg/en/lvb/l28130.htm

Jank, B. and Gangitsch, H. (2001). Decision making under the Cartagena Protocol on Biosafety. *Trends in Biotechnology Journal* 194-197.

The use of genetically modified animals. The Royal Society. (2001). www.royalsoc.ac.uk/displaypagedoc.asp?id=11513

Participant Discussion

Johnsson: Have your field trials in Hungary been published?

Maclean: Yes. (See Journal of Fish Biology (2001). 59: 62-78, Rahman et al). The main outcome was that the tilapia were growth enhanced two and a half fold in seven months without any obvious abnormalities. Further work was done on muscle quality; in general they were very nice fish. The only thing different is that you might notice that the head is a slightly different shape. After about ten generations, nothing other than that has been noticed. We do know that this strain of transgenic tilapia make approximately twice as much growth hormone gene as non-transgenic tilapia. They produce as much salmonid growth hormone as they do their own. We have purposefully selected low hormone producing strains for production so as not to have over selected production side effects.

Kapuscinski: The facility in Hungary appears to be an interesting example of environmental confinement. Could risk assessments be done in this facility?

Maclean: This paper is published in the Journal of Fish Biology, but does not give much information on the situation in Hungary or the facilities used. There are many ponds, the staff are very professional, the facilities are top notch and the fish were well contained.

Dunham: What type of ponds were being used, are they earthen?

Maclean: They were huge fiberglass cages (huge heat exchanger used to heat the water, very expensive). The original plan was to use the water from underground, but it was poor quality and so instead it was used to heat good quality water using a heat exchanger. There are concrete walls around the facility and overhead protection because of predation.

Poon: Are there, or will there be, any trials in Thailand using the modified tilapia?

Maclean: They have contained ponds there and this is more accurate representation of the way they are normally cultured. This has not happened as of yet, but we are in negotiations and this option is currently being explored.

GENETIC MODIFIED FISH RESEARCH IN CUBA

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Abstract

The genetic modification of aquatic organisms has become an important tool to improve the productivity of aquaculture and mariculture. Recently, we have obtained a transgenic tilapia line with accelerated growth. However, before introducing this line into Cuban aquaculture, environmental and food safety assessment was required by national authorities. Experiments were performed to evaluate the behavior of transgenic tilapia in comparison to wild tilapia as a way to assess the environmental impact of introducing transgenic tilapia into Cuban aquaculture. Studies were also conducted to evaluate, according to the principle of substantial equivalence, the safety of consuming transgenic tilapia as food. Finally we are conducting an experiment to evaluate the composition of nutritionally and physiologically important molecules in transgenic tilapia compared with non-transgenic tilapia and their impact in the mammalian gut. Behavior studies showed that transgenic tilapia have lower feeding motivation and dominance status when compared to wild tilapia. Food safety assessment indicated that tilapia growth hormone has no biological activity when administered to non-human primates. Furthermore, no effects were detected in healthy human volunteers after the consumption of transgenic tilapia. These results showed, at least under the conditions found in Cuba, no environmental implications for the introduction of this transgenic tilapia line and the safety in the consumption of tiGH-transgenic tilapia as an alternative-feeding source for humans. These results about nutritional value and physiological studies of genetically modified tilapia support their culture and consumption.

Résumé

La modification génétique d'organismes aquatiques est devenue un outil important pour améliorer la productivité de l'aquaculture en eau douce et en eau salée. Nous avons récemment obtenu une souche de tilapias transgéniques à croissance accélérée. Cependant, avant que cette souche ne soit utilisée en aquaculture à Cuba, les autorités nationales exigeaient une évaluation environnementale et une évaluation de la salubrité des poissons de cette souche. Des études ont été menées afin d'évaluer le comportement des tilapias transgéniques par rapport à celui des tilapias sauvages dans le but d'évaluer les effets environnementaux de l'utilisation de tilapias transgéniques en aquaculture à Cuba. Des études ont également été menées afin d'évaluer, conformément au principe d'équivalence substantielle, la salubrité des tilapias transgéniques pour la consommation. Finalement, nous réalisons une expérience visant à évaluer la composition de molécules

importantes sur le plan nutritionnel et physiologique présentes dans les tilapias transgéniques par rapport à celle dans les tilapias non transgéniques ainsi qu'à évaluer les effets de ces molécules dans l'intestin de mammifères. Des études sur le comportement ont montré que les tilapias transgéniques ont une stimulation alimentaire et un statut de dominance inférieurs à ceux des tilapias sauvages. L'évaluation de la salubrité a indiqué que l'hormone de croissance du tilapia n'a aucune activité biologique dans le corps de primates non humains. De plus, aucun effet n'a été détecté chez les volontaires humains en santé qui ont consommé du tilapia transgénique. Ces résultats montrent que, à tout le moins dans les conditions qui prévalent à Cuba, l'utilisation de la souche de tilapias transgéniques ne présente aucun risque pour l'environnement et que ces poissons sont salubres et peuvent être utilisés par l'homme comme source de nourriture de substitution. Ces résultats à propos de la valeur nutritive des tilapias génétiquement modifiés, de même que des études physiologiques sur ceux-ci, appuient leur élevage et leur consommation.

Introduction

The tilapias are a group (about 70 species) of African freshwater herbivorous fish. They have a number of attributes ideal for aquaculture, such as a quick growth rate, few bones, fresh taste, good market acceptance, ease of reproduction, and of course, adaptability to a very wide range of environmental conditions.

In the last 50 years, tilapias have been introduced in many countries of the world and they are probably the most widely cultures fishes in the tropics. The quantities of tilapia now being produced through aquaculture are growing rapidly. Tilapias are well on the way to becoming the aquatic version of the chicken because they can be mass produced from eggs, can be farmed highly profitably in a variety of situations from backyard enterprises to high-technology systems, and can convert plant products into animal protein at a very efficient and economical 2:1 ratio.
Therefore tilapias, like chickens, are a source of low-priced animal protein. These species need around 6 months to get the commercial weight of 350 g (Tave 1993), thus constituting species of choice to select for strains with improved growth performance.

For developing countries, as Cuba, the availability of animal protein for human consumption is limited; therefore it is the very high importance of research on this topic.

Cuba has developed aquaculture capacities in the last 20 years to increase, in the future, their production up to 100 000 ton. In this sense, new technologies have been introduced and developed in Cuban aquaculture, such as genetically improved species, monosex population procedure, sperm cryoconservation, intensive, and super intensive culture.

We have an improved transgenic tilapia line that grows faster than wild type, reaching commercial weight in only four months, which would mean almost duplication of our production capacities (Martínez et al. 1996).

On the other hand, the current world public perception and the acceptance of GMOs are much discussed. There are two major focuses in the present debate: human health and ecological impact of GMOs. We have done several experiments to characterize our transgenic tilapia that have suggested up to now its safety; both to our intensive aquaculture system and as a human food.

Taxonomy of the GM fish

At the end of the eighties the Center for Genetic Engineering and Biotechnology (CIGB) of Havana, Cuba, decided to introduce the transgenesis in fish as a project with the aim to improve the growth performance in the tilapia culture. The Cuban Ministry of Fisheries gave a genetically improved hybrid tilapia to use as a target to obtain transgenic tilapia.

The hybrid tilapia used for trangenesis is from the cichlidae family, *Oreochromis genera* and a hybrid species between *Oreochromis aureus* and *Oreochromis hornorum*.

Design of trangene constructs and pattern expression

In the last few years, many experimental approaches have been shown to increase the growth rate of fish (Mommsen 1998). Among other approaches it has been established that growth hormone (natural or recombinant) administration promotes the growth acceleration in fish (Guillen et al. 1998). Growth manipulation through transgenesis was the choice methodology by the CIGB Havana. Transgenic tilapia lines were generated by the transfer of chimeric genes containing the tiGH cDNA under the regulation of the cytomegalovirus (CMV) or Rous sarcoma virus (RSV) promoters (de la Fuente et al. 1995; Hernández et al. 1997; García del Barco et al. 1994) and SV40 polyadenilation signal (de la Fuente et al. 1995) (Table 1). Transgenic lines with lower ectopic tiGH mRNA levels are the only ones showing growth acceleration, suggesting a transgene-dosage effect (Hernandez et al. 1997; Martinez et al. 1999) (Table 1). These results are consistent with the reports by groups working with different GH transgenes where they have found a high growth promoting activity only with relatively weak promoters (Zhang et al. 1990; Zhang et al. 1998).

Table 1. Design of trangene constructs and pattern expression. All constructs carrying the cDNA of tilapia GH (tiGH) and the SV40 polyadenilation signal (Hernández et al. 1997; de la Fuente et al. 1995; Martínez et al. 1996)

Promotor	Copy number/cell[b]	Ectopic transgene expression (RNA)[c]	Growth acceleration (%)[d]
RSV + INT[a]	>50	240	0
CMV – INT	8-30	23	0
CMV + INT	10-30	8	3.4
CMV – INT	1	5	82
Control	0	0	-

[a] *INT: First intron from the trout GH gene*
[b] *Determined by dot blot analysis of fin DNA*
[c] *Determined by in situ hybridization*
[d] *Determined by communal growth trial*

Phenotype characterization of the transgenic tilapia line

The transgenic tilapia carrying one copy of the CMV-tiGH transgene was selected to study the phenotype and inheritance and it was created as a transgenic tilapia line called IG-91/03F70 (Martínez et al. 1999).

The transgenic tilapia line IG-91/03F70 transmitted the transgene in Mendelian fashion, thus indicating that the transgene was stably integrated into the host genome in the early stages of embryonic development. Pedigree analysis of the transgenic tilapia up to fourth generation (F_4) was made (Martínez et al. 1999).

Several communal growth trials have been developed with the transgenic tilapia line IG-91/03F70 (Martínez et al. 1996; Hernández et al. 1997; Cabezas et al. 1998). The results have shown the maintenance of the accelerated growth performance and the growth rate in the phenotype of the transgenic tilapia through the fourth generation including in intensive culture condition (Martínez et al. 1996; Hernández et al. 1997; Cabezas et al. 1998).

Biochemical studies

The enhanced growth produced by the ectopic expression of tiGH in this transgenic tilapia could be due to increased food consumption and/or improved food conversion. This is a fundamental question for biological studies and for cost-effectiveness analysis.

To differentiate these two possible mechanisms, the total food consumption was carefully recorded. When relative food consumption rate was calculated, it was found that transgenic tilapia, when compared to non-transgenics, had a lower food consumption rate (Martínez et al. 2000). Furthermore, the food conversion efficiency was increased by 290% (Martínez et al. 2000). Transgenic tilapia showed a higher protein synthesis rate and lower ration consumption, resulting in higher efficiency of growth, synthesis retention, and anabolic stimulation.

Therefore, this transgenic tilapia is metabolically more efficient, capable of supporting growth with better food conversion efficiency. Krasnov (1999) has reported similar results in rapidly growing transgenic Arctic char. They found specific growth rate and muscle protein content equal with respect to non-transgenic siblings.

The GH exerts its growth-promoting action through different metabolic pathways. There were differences in free alanine and aspartic acid levels in the muscle of juvenile transgenic tilapia (Martínez et al. 1999). An increase in the GOT and GPT transaminases was found at this stage of life in transgenic fish, but not in lactate dehydrogenase enzyme activity, neither in the lactate and glucose levels in muscle tissue (Martínez et al. 2000). Transgenic juvenile tilapia had lower hepatic glucose and a higher piruvate kinase activity, showing an enhanced glucolysis when compared to non-transgenics (Martínez et al. 2000). There were no differences regarding the levels of lactate and glucogen, neither in the hepatosomatic index between transgenic and non-transgenic tilapia (Martínez et al. 2000). Although these results reflect a metabolic unbalance in the liver of juvenile transgenic tilapia, the maintenance of the hepatosomatic index denotes that this unbalance is probably within physiological levels.

The total contents of RNA, DNA, and protein were measured in juvenile and adult muscle of transgenic and non-transgenic tilapia. No differences were found except in the RNA/DNA and RNA/protein index in transgenic juvenile and adult muscle, respectively (Martínez et al. 2000). However, the difference in the RNA/DNA ratio in juvenile tilapia was not statistically significant and represented only a tendency. This result reflected an increase in the protein synthesis in this tilapia. Similar results have been reported for GH-treated fish (Sun and Farmanfarmaian 1992 a,b). In adult transgenic tilapia, an increase in the RNA/protein ratio reflected an effect of ectopic tiGH on ribosomal capacity.

Biochemical analyses in adult tilapia showed no differences between transgenic and non-transgenic animals (Martínez et al. 2000). This result is important for the evaluation of the possible effects of consuming transgenic tilapia as it further documents that transgenic tilapia IG-91/03F70 is safe as food (Guillén et al. 1999).

Tilapia ions profile was studied for transgenic and non-transgenic tilapia white muscle, and there were no differences found between them (Manuscript in preparation).

As growth hormone exerts a variety of effect on diverse metabolic processes including lipid metabolism, a quantitative analysis of the non-sterified fatty acids composition was performed in white muscle samples from transgenic and non-transgenic tilapia. There are higher amounts of unsaturated fatty acids (16:1; 18:1; 20:2; 20:4; 22:6) and lower amount of saturated fatty acids (16:0; 22:0; 24:0) compared with non-transgenic one (Manuscript in preparation).

Evaluation of the ecological impact of the introduction of transgenic tilapia line* IG-91/03F70 *with accelerated growth in Cuba

To conduct the studies with transgenic tilapia outside the laboratory, we held an *ad hoc* committee meeting devoted to the analysis of the conditions to release transgenic tilapia with accelerated growth in Cuba (de la Fuente et al. 1996). This committee concluded that, under the conditions found in Cuba, little or no effect on natural populations will occur as a result of accidental escape or intentional introduction of transgenic tilapia. This is mainly because these natural populations do not exist and most of the fish species we found now in the country have been introduced. Nevertheless, the committee recommended to follow the final draft of the "Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish" (documents No. 95-01 and 95-02) prepared and released by The U.S. Department of Agriculture, the Agricultural Biotechnology Research Advisory Committee, and the Working Group on Aquatic Biotechnology and Environmental Safety (de la Fuente et al. 1996; Guillén et al. 1999). Our experiments have been planned and conducted following these recommendations.

The accessible ecosystems contain co-specific species with which transgenic transgenic tilapia could breed. Since transgenic animals are fertile, there is a risk for a potential introgression of the chimeric tilapia growth hormone gene into natural population. Nevertheless, in the case of Cuba, tilapia was introduced in the sixties and there is no risk of affecting endogenous species. We are dealing with an ecosystem deliberately modified.

The National Center for Biological Biosafety (NCBB) was created in 1996 and is the national regulatory authority in charge of licensing of studies and products focusing mainly on the biosafety of the ecosystem. There are rigorous rules to follow at each step of any research project invlolving GMOs. The regular guidelines to grant a license for commercialization in Cuba have been reviewed very carefully, but are still unregulated by the NCBB. Aside from that, approval for use as food is from the Regulatory Bureau which is part of the National Ministry of Public Health. These regulations are also undergoing revision and have not yet been released by the Regulatory Bureau.

Because we do not have all the information, we decided to evaluate behavior, reproductive potential, and other parameters that could give information about this genetically modified organism.

Behavior studies

Behavior studies with transgenic tilapia and in comparison with wild type tilapia were selected to evaluate the possible impact on wild tilapia populations of accidentally escaped transgenic tilapia.

Feeding motivation and dominance status (DS), in wild type tilapia were higher when compared to transgenic tilapia. However, transgenic tilapia showed a higher avidity for the food when compared to non-transgenic siblings. Similar results were obtained by Johnsson and Björnsson (1994) in juvenile rainbow trout injected with GH. These findings may indicate an effect of ectopic tiGH expression on these parameters.

Osmoregulation

Transgenic tilapia showed a better osmoregulatory capacity when compared to non-transgenic siblings and wild type tilapia. These results suggested that GH could be involved in osmoregulation in tilapia, given an advantage for seawater adaptation of transgenic vs. non-transgenic tilapia. However, it is generally accepted that tilapia, even if they survive in seawater, are unable to reproduce or their fertility is severely impaired (Guillén et al. 1999).

Morphological and meristic characterization

Although transgenic tilapia have been manipulated to accelerate growth, many characteristics of the parental organism should be maintained. We have not seen any abnormalities in transgenic tilapia (Morales et al. 1998 a,b).

These preliminary observations addressed some of the concerns discussed by Hallerman and Kapuscinski (1992) for the introduction of transgenic fish into national aquaculture programs, and provide evidence that, at least under the conditions found in Cuba, no ecological impact could be anticipated for the introduction of transgenic tilapia to national aquaculture.

Metabolic rate and exercise performance

Swimming respirometry was employed to compare inactive metabolic rate, maximum metabolic rate, resultant aerobic scope and maximum sustainable (critical) swimming speed, in the transgenic tilapia and wild-type tilapia *Oreochromis* sp. hybrids. Although metabolic rate was significantly higher (58%) than their control conspecifics, there were no significant differences in their net aerobic scope. As a consequence, the two groups had the same maximum sustainable (critical) swimming speed. The transgenic and wild type tilapia also exhibited the same capacity to regulate oxygen uptake during progressive hypoxia, despite the fact that the transgenic fish were defending a higher demand for O_2. The results indicate that ectopic expression of GH raises metabolic rate in tilapia, but the

fish compensate for this metabolic load and preserve such physiological determinants of fitness as aerobic scope, swimming performance, and tolerance of hypoxia (Mckenzie et al. 2003).

Evaluation of the reproduction

The reproduction capacity of the most important tilapia species (*O. niloticus* and *O. aureus*) in the Cuban aquaculture system was compared with transgenic tilapia. In the same conditions used in aquaculture larvae production ponds, the results have shown that transgenic tilapia have better reproduction capacity (larvae/m2; larvae/female and larvae/g of female) than the species compared, except regarding larvae/g of female when *O. niloticus* females were used (Manuscript in preparation).

Evaluation of human health risk

Evaluation of the effect of the recombinant tiGH on mammals

It was demonstrated that the recombinant tiGH did not stimulate sulfate uptake in rabbit cartilage (Guillén et al. 1999), suggesting non-recognition by mammal GH receptor of this fish protein.

In vivo activity of tiGH administered to non-human primates

Based on the principle of substantial equivalence (Jonas 1996; Guillén et al. 1999), we designed an experiment to determine if recombinant tiGH was capable to exert some modifications on somatic growth parameters and/or in blood and serum profile using a short-term administration regimen in non-human primates. The results show that tiGH did not lead to modifications in animal behavioral pattern, body weight, respiratory rate, body temperature, serum and blood profiles, and somatometric and morphological parameters (Guillén et al. 1999).

Evaluation of the consumption of transgenic tilapia by human healthy volunteers

The possible hazardous effects associated to human consumption of genetically manipulated fish bearing GH transgen have not been assessed so far. For this purpose, we designed a randomized blinded trial employing healthy human volunteers.

The results of this trial showed that transgenic tilapia flesh was well accepted with a better flavor evaluation. Furthermore, no differences were recorded in the clinical and biochemical parameters evaluated before and after the onset of the experiment, neither between experimental groups. (Guillén et al. 1999).

Nutritional value

Feeding studies of diets containing as protein source the white muscle of the transgenic and non-transgenic tilapia were conducted in rats, in order to know several parameters as digestibility, biologic value, net protein utilization, and protein efficiency rate. During the period tested, rats in each group grew well without differences in appearance and body weight; no significant differences were also found regarding the mentioned parameters studied between them. The results can be considered satisfactory since it is generally considered that a protein with a biological value >70 % is able to meet nitrogen requirements during growth (Gonzalez et al. 2001). Necropsy at the end of the experiment indicated neither pathologic symptom in all rats tested nor histopathological abnormalities in the intestine. Judging from these results, the transgenic tilapia fillet is confirmed to have nearly the same nutritional characteristics as non-transgenic one (Manuscript in preparation).

Conclusion

Transgenic tilapia, line IG-91/03F70, proved to be a good phenotype for aquaculture and was shown to be safe for both human health and ecosystem. It showed a better growth and reproduction capacity than non-transgenic tilapia and caused no detectable health or environmental complications, at least under the conditions assayed.

Up to now there are not any biological characteristics damaged by the transgenesis in these genetic modified fishes. However, other experiments should be conducted to better understand the relationship between the GM fish and the environment.

It is necessary before release of GM fish to a production system to assess performance by the Net Fitness approach suggested by Muir et al. (2000), in the Pew Fellowship Project (This approach requires data on six fitness factors for the genetically modified organism and unmodified controls: viability, fecundity, fertility, mating success, age at sexual maturity, and longevity). Moreover, it will be important to develop a study to assess the possible level of allergenecity of the transgenic fish. Further, once the national institutions have devised the regulations required for the proper assessment of transgenics, a lengthy process of evaluation will follow.

Biotechnology in GM fish is close to giving powerful final results to increase the aquaculture efficiency. It is very important to take into account that people worldwide eat more fish than any other type of animal protein and the increasing of the global population.

Although each country must consider their specific situation, the introduction of transgenic fish strains should be generally accepted but it is necessary to overcome scientific and social barriers.

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Participant Discussion

Maclean: What do you think the future is for this technology in Cuba? Will there be commercial production, or will this be a while?

Martinez: My wish is that it will be soon, but the regulations are quite tough and regulations match the European model, and thus it is not likely to go commercial for quite some time. The answer is yes, but not in the near future.

Kapuscinski: Could you clarify the data that osmoregulation is improved by transgenics?

Martinez: We would like to do this sort of work in sea cages. Previous results say that in seawater Tilapia cannot reproduce, so we would like to know with this gene construct if that is also the case.

Dunham: Is one of the construct-containing or hybrid species more saltwater tolerant?

Martinez: This is not known.

Dunham: What type of feed were you using? The 9.7 Food Conversion Efficiency (FCE) for controls is about 4-5 times higher than one might expect. Why do you think this might be?

Martinez: Due to using feed pellets. We used the same feed and the same methods for both controls and transgenics.

Dunham: Normally for controls you would expect an FCE of about 2.0 but these food conversion rates seem inordinately high in comparison to most literature data. (**Statement).**

Dunham: Wild type tilapia (*O. aureus*) take more pellets than the transgenic hybrids. Since the wild type is eating more pellets, is the wild type growing faster than the transgenics?

Martinez: No.

Benfey: Can you explain the osmoregulation data? The data looks like they are saying the transgenic fish are different before 50% saltwater exposure, but not after. Before, were the fish in freshwater? Did you only look at 3 fish of each type for this data?

Martinez: Yes.

Donaldson: How would you deal with exports of this fish?

Martinez: I am not the right person to answer this. But there is no commercial production right now. This will likely be addressed later by the Cuban regulatory agencies.

Kapuscinski: Cuba signed the Cartagena strategy, so this will likely determine how they will proceed with exports. (**Statement**)

Donaldson: Cuba is quite well contained. Not really any risks of escapes. (**Statement**)

ACADEMIC USES OF GM FISH TECHNOLOGY: A CALL FOR A COMMON SENSE APPROACH TO REGULATION

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Abstract

There are 8 broad categories of uses for GM fish: (1) to determine the function of normal genes, (2) to study the genetic basis of disease, (3) to provide cells for xenotransplantation, (4) to develop and produce therapeutic proteins, (5) to improve methods of testing chemicals, (6) to improve methods of genetic modification in a particular species, (7) to study the potential environmental impact of the accidental release of GM fish from fish farms, and (8) to improve fish farm productivity (not discussed here, but covered in the remainder of this proceedings). Based upon a comprehensive review of the Entrez-PubMed database citations (1986-2003), it is clear that research on transgenic fish is increasing exponentially, that the vast majority of transgenic fish research relates to warm water species (e.g., zebrafish, medaka, tilapia, etc.) that could not live outside of indoor laboratories in Canada (i.e., accidental escape does not pose environmental problems), and that the vast majority of transgenic fish use pertains to developmental or medical research (and not fish farming). Finally, I review the current status of transgenic fish regulation in Canada and make a strong plea to avoid future over-regulation of the academic use of warm water transgenic fish.

Résumé

Il existe huit grandes catégories d'utilisations des poissons génétiquement modifiés : 1) déterminer la fonction de gènes normaux; 2) étudier la base génétique de maladies; 3) fournir des cellules aux fins de xénotransplantation; 4) élaborer et produire des protéines thérapeutiques; 5) améliorer des méthodes d'essai de produits chimiques; 6) améliorer les méthodes de modification génétique de certaines espèces; 7) étudier les répercussions environnementales possibles de la libération accidentelle de poissons génétiquement modifiés élevés dans une ferme piscicole; 8) améliorer la productivité de fermes piscicoles (cette utilisation n'est pas abordée dans le présent document, mais elle l'est dans le reste de ces actes). D'après un examen exhaustif des citations de la base de données PubMed® (1986-2003), il est clair que le nombre de recherches axées sur les poissons transgéniques croît de façon exponentielle, que la grande majorité de ces recherches portent sur des espèces des eaux chaudes (p. ex. poisson zèbre, medaka, tilapia, etc.) qui ne pourraient pas survivre à l'extérieur des laboratoires au Canada (c.-à-d. une évasion accidentelle ne présente aucun risque pour l'environnement) et que la grande majorité des utilisations des poissons transgéniques ont trait à des recherches sur le développement ou à des recherches médicales (et non à la pisciculture). Finalement,

j'examine l'état actuel de la réglementation relative aux poissons transgéniques au Canada et je plaide fortement en faveur de la prudence afin d'éviter une surréglementation de l'utilisation de poissons transgéniques d'espèces des eaux chaudes dans le contexte universitaire.

Genetically modified (GM) fish are clearly a "risky business". Much has been written, in the midst of much uncertainty, about the potential risk of environmental damage if GM fish were to be accidentally released into the environment; intuitively, in the context of GM crops such as rapeseed and wheat blowing their ways unimpeded across the Prairie Provinces, this seems to be a potential risk that must be taken very seriously. Even more has been written, in the midst of even more uncertainty, about the possible health risks associated with consumption of genetically modified organisms (GMOs). Here the level of risk seems less, but, nevertheless, there are huge potential economic risks for large companies which have invested very large sums of money in GM crops (both plants and animals) without doing due diligence research to determine whether the public would readily accept them; these same companies would like to convince regulators that there is no need to label GM products, essentially hiding them from the public so that consumers have no say in the matter of whether they consume GM crops. In the field of medicine, hiding this information would be considered unethical because it flies in the face of several overarching ethical principles: "respect for persons" (i.e., allowing competent and autonomous people the right of self-determination), "avoiding deception and non-disclosure" and "obtaining informed consent". Finally, there are other important economic and ethical issues related to the proliferation of GM crops; GM crops are somebody's intellectual property and thus are more expensive than "wild type" crops. Therefore, people in developing countries are at potential risk and need to have access to cheaper alternatives that are more in keeping with their economic status. In medicine, we have already seen examples of this; for instance, pork and beef insulins used to be very inexpensively produced by chemical extraction from animal pancreases; now pharmaceutical companies produce only recombinant human insulins from genetically engineered bacteria; because of intellectual property rights, such insulins, are more expensive and less affordable to diabetic patients in developing countries. Thus, there is clearly important environmental, health, economic, and ethical risks associated with GM fish.

These seem to be the overarching risks related to genetic modification of commercially important fish species when the manipulations are done to produce hardy strains of tasty fish that grow rapidly in aquaculture (i.e., farm animals). However, not all GM fish are designed for human consumption or pose any of these risks. The Animal Procedures Committee of the UK has identified six major areas of interest related to the use of GM animals in research and testing (APC Report on Biotechnology). These are: (#1) to determine the function of normal genes, (#2) to study the genetic basis of human or animal diseases with a view to improving the management of disease, (#3) to provide organs for xenotransplantation, (#4) to develop and produce therapeutic proteins, (#5) to

improve methods of testing chemicals, and (#6) to improve production from farm animals. To their list, I would add two others: (#7) studies performed to develop or improve methods of genetic modification in a particular species, and (#8) studies performed specifically to address the potential environmental impact of the accidental release of GM fish from fish farms. This makes a total of eight types of GM animal studies, all of which are applicable to transgenic fish.

Based upon data from a search of the National Center for Biotechnology Information-National Library of Medicine Entrez-PubMed database (http://www.ncbi.nlm.nih.gov/PubMed/) (see Figures 1-3), several important observations pertaining to transgenic fish publications can be made. First of all, as shown in Figure 1, the amount of research on transgenic fish has increased markedly, especially in the past five years. Second, the vast majority of transgenic fish research worldwide relates to tropical or other fish species that could not survive outside of indoor laboratories because of the cold Canadian climate. Figure 2 shows that research with transgenic zebrafish is predominant, but that considerable work is done with medaka, goldfish/carp, tilapia, loach, and other species; transgenic species that could readily survive in Canada are the salmonids, including Arctic char. Third, Figure 3 shows that the nature of transgenic fish research has changed greatly over time. In the mid- and late 1980s, there were only a handful of publications; not surprisingly, the bulk of transgenic fish research at this time related to developing and improving methods of transgenesis with the idea of producing strains of hardy, rapidly growing edible GM fish which would thrive in an aquaculture setting. Throughout the 1990s, technical improvements continued to be published and, by the end of the decade, the uses of transgenic fish had expanded. Because of public sensitivities to GMO foods and because of environmental concerns, there appears to be decreasing research interest in eating GM fish; in stark contrast, interest in GM fish as an animal model for medical research has grown, especially since 1999.

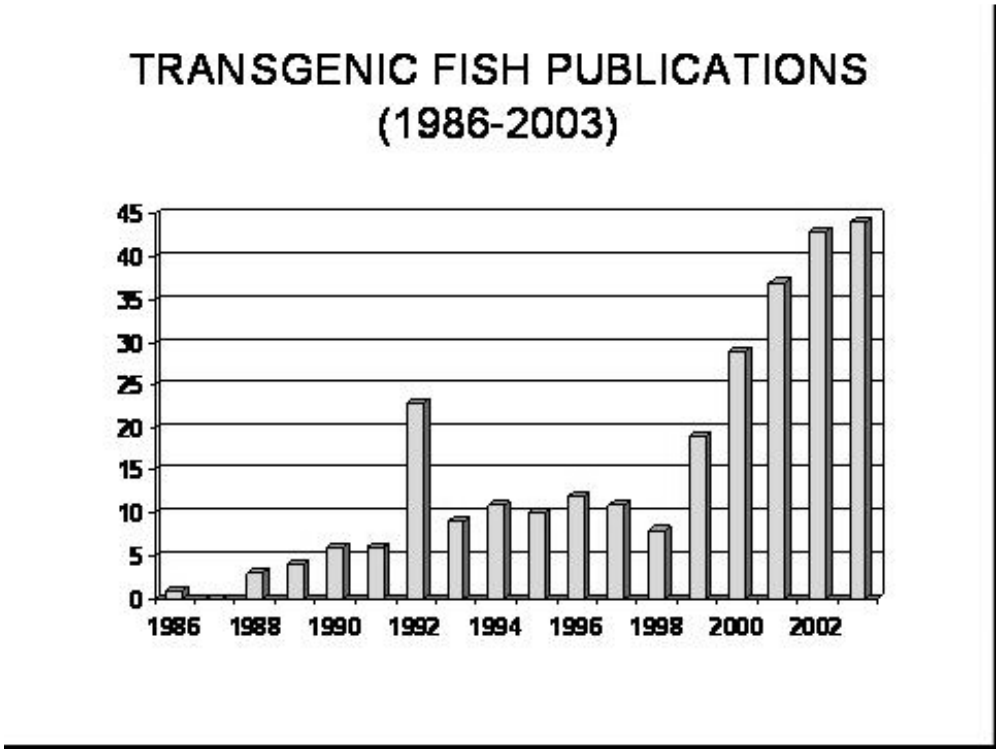

Figure 1. This figure shows the trend for transgenic fish publications based upon data from a search of the National Center for Biotechnology Information-National Library of Medicine Entrez-PubMed database. Note that the number of publications per year remains relatively stable throughout most of the 1990s and then exponentially increases. It should be noted that this figure was generated based upon a literature review in early 2004, and, so, it is likely that the total number of transgenic fish publications for 2003 is incomplete as the entry of citations from obscure journals tends to lag behind that of more mainstream journals. The aberrant spike in 1992 represents a series of articles in a single journal theme issue on transgenic fish.

This trend should not be surprising and can be safely projected to continue. Using data for all animal research in the UK in 1992 vs. 1999, the total number of normal animals used in research decreased by 29%, while the total numbers of GM animals used for research increased by 592%; in comparison, the use of animals with naturally occurring mutations increased by only 44% in that same time period. Zebrafish and medaka are approaching this same crossroads; currently, there are large numbers of naturally occurring mutant strains and relatively smaller numbers of transgenics. However, the availability of GM strains is increasing very rapidly. Furthermore, once embryonic cells (ES), stem cell and gene knock-out technologies have been perfected in fish, these numbers will really skyrocket.

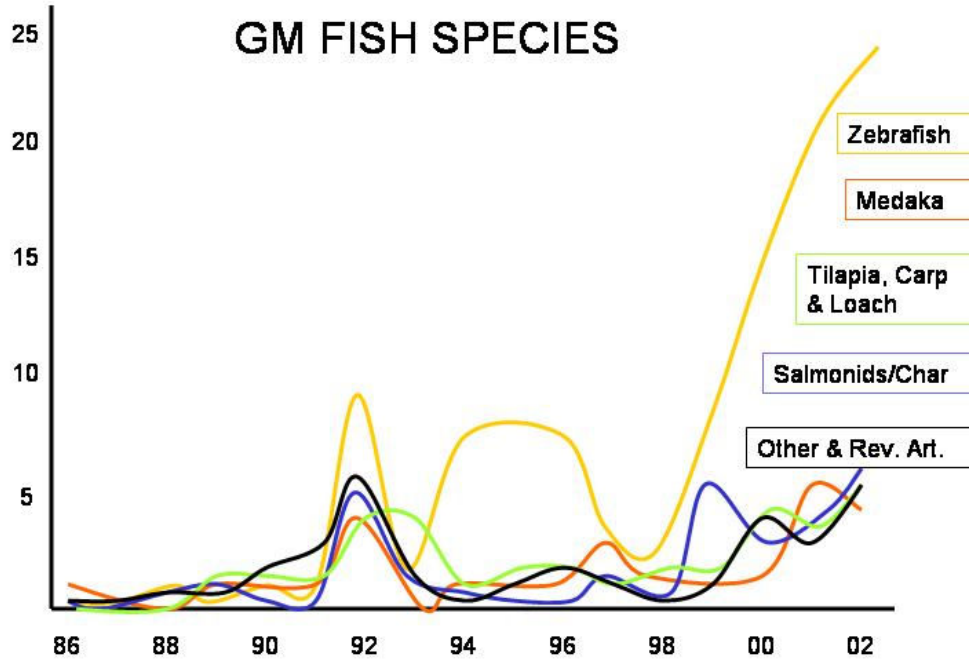

Figure 2. This figure shows the trends (using the same data as in Figure 1) of the various types of fish used in transgenic research. Note that the vast majority are zebrafish and other warm water species; only the salmonids (including char) would seem to raise environmental concerns if accidentally released in Canada.

Currently, most transgenic fish research worldwide is directed at a better understanding of human development and/or gene regulation. Most of this work has been done in zebrafish or medaka. This literature is huge and I will only cite a few recent review articles describing work in the areas of molecular embryology, teratology, neuroscience, apoptosis, and cancer; transgenic zebrafish, often expressing GFP, have proven particularly useful to study the development of the central nervous system, eye, blood vessels, blood, pancreas, liver, and gonads (Tomasiewicz et al. 2002; Field et al. 2003a; Udvadia and Linney 2003; Hsu et al. 2001; Yamashita 2003; Stern and Zon 2003; Field et al. 2003b; Ober et al. 2003).

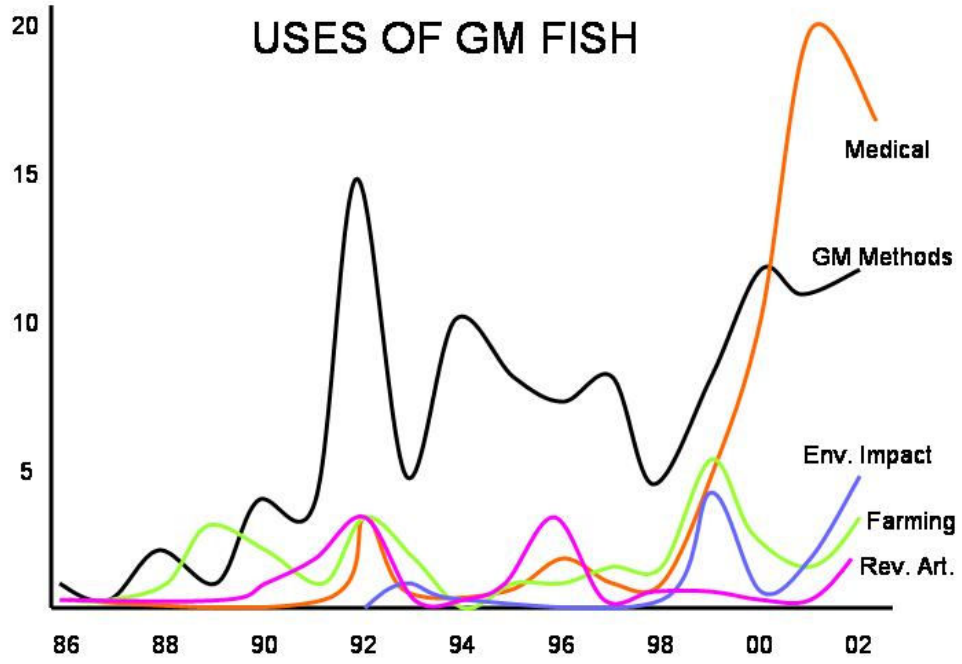

Figure 3. This figure shows the topical trends in transgenic fish research (using the same data as in Figure 1). Note that the vast majority of publications relate to medical or developmental biology research and improvement of GM methods; research directed at improved aquaculture represent only a tiny fraction of all GM fish research.

Regulating GM fish produced for medical or scientific research purposes (i.e., laboratory animals) is illogical. Since these fish are not housed outside, the risk of accidental release is minuscule. Furthermore, the most important transgenic fish species used for medical research in Canada (zebrafish, medaka, and tilapia) are all tropical species that could not escape and survive in Canada. There is currently no specific mandate for federal regulation of the production and use of other types of genetically engineered laboratory animals in Canada (n.b., see discussion of CEPA's New Substances Notification Regulations below). The situation pertaining to laboratory research with tropical fish species that could not even theoretically survive in the harsh Canadian environment would seem to be much more secure than that of genetically engineered mice and rats, which could clearly escape and survive. Admittedly, most GM mice and rats, raised in specific pathogen free environments, are probably not as vigorous as their feral counterparts; thus, escapees probably would not survive long enough to add their genetic distinctiveness to the wild-type murine gene pool. Furthermore, if a murine "Trojan gene" was introduced into the wild; its effect would undoubtedly be minimal because of the vast population of wild type mice (i.e., in stark contrast to the situation with wild salmon populations).

Regulation of the production and use of genetically engineered laboratory animals already occurs very effectively at multiple levels in an academic environment. First of all, there is an absolute requirement to have the written approval of local institutional animal committees before any animal research can be conducted on any animals in any Canadian university or university hospital. Second, there is very effective regulation of the scope and purpose of all animal research by granting agencies, institutional animal use committees, institutional research offices, and the Canadian Council on Animal Care (CCAC); although each has a slightly different agenda, linkage creates a very effective safety net by controlling the acquisition and utilization of research funds as well as access to and use of animal facilities. Institutional animal committees within universities do not approve the use of research animals in research that has not been validated by peer-review. Usually, this is in the form of a research grant application sent to a national funding agency which describes the scope and the aims of the proposed research. Because of limited funding available from these agencies, the usual process is that these research proposals are highly scrutinized by both external and internal reviewers who then rate each proposal, determining whether it is funded. This review process evaluates not only the quality of the science, but also whether the work is likely to produce "added value" to society. Until fairly recently, this was how almost all research in universities was funded. Now there is an additional minor pathway – industrial funding, which often occurs when an industrial partner perceives that certain research may generate valuable intellectual property. Sometimes industrial research is more in the form of a contract to test the safety or efficacy of a product. However, even such research/testing, if performed in a university-based facility, must be reviewed by the university's institutional animal committee as to whether it meets local and national animal ethics standards and must also be peer-reviewed to assess the scientific validity of the work (because of the confidential nature of some industrial research, this process must be handled confidentially). Even if the research is both funded and conducted external to the investigator's own university, a university-based researcher must generally either obtain ethical approval from his/her institutional animal committee or provide a copy of approval from the institutional animal committee at the external site where the work is to be performed. Institutional research offices administer research grants and accounts. Therefore, the use of any animals for research in Canada is already highly regulated.

The CCAC is responsible for the oversight of animals used in research, teaching, and testing in Canada. The CCAC, whose primary concern is the ethical care and use of animals, recognizes that transgenic animals are different from other laboratory animals and, thus, has published specific guidelines in 1997 pertaining to research with transgenic animals (CCAC, 1997). According to the CCAC, these guidelines were created specifically "to assist Animal Care Committee (ACC) members and investigators in evaluating the ethical and technological aspects of the proposed creation, care and use of transgenic animals; to ensure that transgenic animals are used in accordance with the CCAC statement *Ethics of Animal Investigation*; and to ensure that the well-being of Canadians and the environment are protected." These guidelines address: proposals to

create new transgenic strains, proposals to utilize existing transgenic strains, accounting for the numbers produced or used, the need for researchers to be able "to justify their work as being in the public interest", and, finally, appropriate containment of transgenic animals. Pertaining to the latter, the guidelines require that "risks to human health and the environment are minimized to an acceptable level". The guidelines further segregate transgenic animals produced by retrovirus-mediated gene transfer, which could theoretically pose risks to human health (n.b., this issue would also clearly fall under federal Occupational Health regulations, e.g., WHIMIS), from those "created using microinjection or replication-defective viruses (where) the containment risks are limited to those associated with the escape of the animal and interbreeding with wild stocks". More recently, the CCAC has updated its guidelines on *The Care and Use of Fish in Research, Teaching and Testing* (CCAC, in final draft), which provides several new guidelines related to welfare of transgenic fish, prevents consumption of fish with "human or humanized genomic material", requires that transgenic fish must be housed in "dry land, escape-proof containment", and, as currently written, requires the local ACCs to "ensure that investigators have received approval from DFO" (n.b., this last guideline, as related to warm water species, is currently not entirely congruent with federal regulations, see below). It is also important to note that the CCAC also regulates industrial use of animals in research and testing within Canada.

In addition to research uses, other potential medical uses of transgenic fish are as commercial sources of proteins, drugs, or cells for the treatment of human diseases. Transgenic fish (like transgenic pigs, sheep, and cows) can be used as bio-reactors for large-scale production of human proteins such as clotting factors or alpha-1-antitrypsin (Chen et al. 1995). Recently Maclean et al. (2002), in collaboration with Aquagene Inc. (Alachua, FL, USA), have made transgenic tilapia producing human factor VII (Maclean et al. 2002). Very recently, Professor Gong Zhiyan and researchers at National University of Singapore announced that they had genetically engineered zebrafish to produce very large quantities of hepatitis B vaccine (they estimate 1 kg of fish muscle could produce 27 g of vaccine); although this is a significant accomplishment, transgenic zebrafish are probably too small to make very useful bio-reactors and I presume they will replicate this work is a larger species such as tilapia. Transgenic fish can also be used as a source of cells for experimental or clinical xenotransplantation (Wright et al. 2004). Our laboratory has recently produced transgenic tilapia expressing a "humanized" insulin gene in the Beta cells of their pancreatic islets which we hope to use, after extensive characterization, as a source of transplantable cells for the clinical treatment of diabetes (Pohajdak et al. 2004). For any of these kinds of applications, regulation during the early or developmental stages is the same as described above for laboratory animals. Once the work has reached preclinical testing or production stages, Health Canada would also tightly regulate every aspect of this work. For xenotransplantation, all aspects of the use of transgenic fish cells as a medical product would fall under regulation by Health Canada (Proposed Canadian Standard for Xenotransplantation 1999). For human products produced in transgenic fish bio-reactors, Health Canada would invoke different statutes.

As shown in Figure 3, two other areas of GM fish research have prospered over the past few years: (1) studies examining the potential environmental impact of accidental release of transgenic fish (reviewed in Muir and Howard 2002) and (2) studies aimed at using GM fish to test for water pollution (reviewed in Carvan et al. 2000; Winn 2001).

In Canada, the regulation of transgenic animals is also under the jurisdiction of Environment Canada. The Canadian Environmental Protection Act (CEPA) provides for regulation of new biotechnology "substances" (including transgenic organisms other than microorganisms) manufactured or imported which are not covered by other federal acts. CEPA's New Substances Notification Regulations require that Environment Canada be notified about "new substances" derived through biotechnology [new substances are defined as those not on the Domestic Substances List which is "based on substances deemed to be present in Canada between January 1, 1984 and December 31, 1986"]; however, Section 29/16 states that organisms other than microorganisms that are "research development substance(s) ... imported to or manufactured in a facility from which there is no release into the environment" are not subject to notification. This section would appear to obviate the need to notify Environment Canada when transgenic fish which could not survive in Canada are being used for scientific research in indoor facilities
(http://strategis.ic.gc.ca/epic/internet/inbravo-canada.nsf/vwGeneratedInterE/h_ak00143e.html).

In this essay, it seems appropriate to briefly discuss the "Glofish" controversy. Glofish are GM zebrafish which were engineered by scientists at National University of Singapore to express genes for colorful fluorescent proteins from sea anemones and coral; their eventual goal is to achieve zebrafish with selective fluorescence when exposed to water pollution/ environmental contaminants. However, capitalists noted a potential niche for constitutively fluorescent ornamental aquarium fish and sales worldwide have been brisk. Since January 2004, Glofish have been marketed throughout the USA (except for California) by Yorktown Technologies (Austin, TX, USA). Based upon extensive information posted on the Glofish website (www.glofish.com), the company appears to have done an excellent job demonstrating that these fish pose no significant environmental or health risks; thus, the US Environmental Protection Agency, the US Fish and Wildlife Service, the US Department of Agriculture, and the FDA expressed no regulatory interest in them. However, on December 4, 2003, California's Fish and Game Commission decided to ban Glofish sales, against the recommendations of its own scientists, citing ethical concerns about genetic engineering for trivial uses. On December 9, 2003, the FDA issued the following statement: "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 danio 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 clear risk to the public health, the FDA finds no reason to regulate these particular fish." This decision angered a coalition of environmental and

food safety groups (Center for Food Safety, the Sierra Club, Consumers' Union, Greenpeace, etc.), which demanded that the FDA intervene and block the release pending a safety review. In mid-January 2004, they filed a suit against the FDA, which has stated it will oversee transgenic fish on a case-by-case basis. In stark contrast to their rapid decision on Glofish, the FDA has been very carefully scrutinizing transgenic salmon from Aqua Bounty Farms (Waltham, MA, USA) for several years. I believe that the FDA has shown wisdom in its decision not to regulate glofish as these clearly fall outside of their mandates. In mid February 2004, Environment Canada banned the importation and sale of Glofish pending formal environmental risk assessment.

What is the object of regulation? Is it to protect the environment and the consumer, or is it to control all fish modification? I believe that there is a need to define the elements of the problem precisely and then to avoid a solution that is bigger than the problem. Since this essay deals with risk assessment, I would like to point out that there are risks associated with over-regulation. First of all, regulation of transgenic tropical fish that pose no apparent risk to the environment and are not designed for human consumption, dilutes human and fiscal resources that could be better directed elsewhere. As shown in Figure 3, the academic use of transgenic fish in medical research, as measured by the annual number of publications in scientific journals, is growing rapidly and there is no reason to suspect that this trend will change. The bio-technology industry worldwide has very extensive proprietary interests in zebrafish technologies including transgenics. For example, Zymogen, LLC (Atlanta, GA, USA) designs transgenic zebrafish with GFP-labeled organs or cell lineages for use in gene characterization, drug development, and toxicological testing. Other companies include: Artemis Pharmaceuticals GmbH (Cologne and Tubingen, Germany), Gene Tools, LLC (Corvallis, OR, USA), Mermaid Pharmaceuticals GmbH (Hamburg, Germany), Phylonix (Cambridge, MA, USA), DanioLabs (Cambridge, UK), Aventis Pharma (Frankfurt, Germany), Discovery Genomics (Minneapolis, MN, USA) (Flanagan 2003; McCoy 2001). Such industries, were they located in Canada, would not want to disclose and have regulated every mutant strain they develop. Over-regulation may prevent innovation and obstruct the establishment of Canadian biotechnology companies. Canada miss out not only on transgenic fish research done by the biotechnology sector, but also on spinoffs such as developing sophisticated indoor aquaculture systems to prevent loss of (or enhance production of) these valuable mutant strains. For instance, if our transgenic tilapia expressing a humanized insulin gene were to be fully commercialized as a xenogenic donor source for pancreatic islet xenotransplantation, we estimate that we would need to produce 45 million transgenic tilapia per year in indoor specific pathogen free breeding facilities for every 100,000 clinical islet xenotransplantation procedures (Wright et al. 2004). If DFO decides to regulate transgenic fish production by bio-technology companies, they will need to establish mechanisms to perform their evaluations with a level of confidentiality and security that will satisfy these companies that their intellectual property rights are being adequately protected (n.b., this has proven to be both problematic and expensive for research offices of Canadian universities that participate in industrially funded research). Activist organizations opposed in principle to transgenic

fish such as Greenpeace or People for the Ethical Treatment of Animals (PETA) could invoke freedom of information legislation to try to thwart this process.

Another danger of over-regulation is that it creates inefficiency and waste. An example of this was our ill-fated Networks of Centers of Excellence-funded study to develop embryonic stem (ES) cell and germ-line chimera technologies in tilapia. Our tilapia hatchery produces grey tilapia. Our initial plan was to make chimeras using blastulas from both red and grey pigmented tilapia. With federal grant funding, we began the process of importing a pure red strain of tilapia (highly characterized with certificates of health) from Brendan McAndrew's laboratory in Stirling, UK in the autumn of 2000. DFO regulatory approval took several months and, because Dalhousie's aquatic facility is a flow through system, required installation of effluent treatment, which then had to be inspected. Once the red tilapia had been imported, each time they were moved between rooms within our aquatic facility, a new permit, installation of additional effluent treatment, and inspections were required. To remove the quarantine (and freely use the blastulas), we were required to raise the juveniles that we had imported to sexual maturity, mate them, raise their offspring to an acceptable size for pathogen testing (i.e., for specific salmonid pathogens) and necropsy studies, and then wait months for the results of these tests. This effectively prevented us from using them during the term of our grant. Although our local DFO office was extremely helpful in obtaining each inspection and permit, we had not anticipated this level of regulation, since the freshwater tropical fish used in our studies could not survive in Nova Scotia if accidentally released (we were actually surprised that importation of tilapia was even regulated as there is apparently little or no regulation when pet stores import tropical fish). To import these "laboratory animals", we were governed by regulations that were designed for importation of salmonids. Although all parties involved recognized that tilapia do not pose any significant risk in Canada, the regulations were followed very precisely (it was unclear whether there was no option to bend the regulations or just no reason for anyone to accept the personal risk associated with making a decision to bend the regulations). Ironically, we now have red tilapia offspring that have passed extensive pathogen screening that we are able to use without quarantine; unfortunately, we no longer have funding or ethical approval from our institutional animal use committee (n.b., ethical approval is dependent upon holding peer-reviewed funding which demonstrates that the quality of the science justifies the animal use). We now have no choice but to pay thousands of dollars per year to maintain the red tilapia colony with no prospect of using them until we have found a new source of peer-reviewed funding; otherwise, we will have to go through this whole process again. Furthermore, during the process of obtaining importation permits for our red tilapia broodstock, it became apparent that we had unknowingly committed multiple regulatory infractions, such as obtaining non-transgenic grey tilapia broodstock from Ontario on multiple occasions without a transfer permit. Although this horror story relates to moving tropical fish into and around Canada in order to do genetic modification (and not GM fish, per se), it is a good example of how well-meaning but inappropriate regulations can stifle research.

I hope I have built a compelling case to take a "common sense" approach to regulating transgenic fish that are not destined for human consumption. I propose the following regulatory framework for research related to the production and use of transgenic fish species in academic institutions in Canada. First of all, it is clear that DFO should be formally notified prior to initiating research involving any GM fish species. If the work involves any indigenous species or any exogenous species that could theoretically survive and reproduce in the harsh Canadian environment, clearly DFO should fully regulate these endeavors. In these instances, written approval from DFO should be required before research offices of academic institutions are allowed to release grant funding to investigators (a similar arrangement exists for institutional animal care committee approval and, thus, this would be easy to implement). However, when the research involves tropical species that could not survive and reproduce in Canadian waters, it seems equally clear to me that DFO should not regulate the production and use of these transgenic fish; this is a waste of time and resources. Academic research involving fish is already effectively regulated at four or more levels (i.e., peer review by granting agencies, approval by institutional animal use committees, institutional research offices, and the CCAC). If tropical fish are to be used for the production of drugs, proteins, or cells for the treatment of human disease, eventually a fifth regulatory body, Health Canada, which has already produced very extensive regulatory frameworks, must become involved. I strongly believe that the DFO should only regulate transgenic fish species in which escapees would be capable of living outside in Canada. Because of the cold climate in Canada, tropical fish species used in medical research are safe; such species should be excluded from formal regulation.

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Participant Discussions

Kapuscinski: In Canada, do you have what we call institutional biosafety committees (IBC's), and can't they take care of this issue [confinement]?

Wright: As far as biosafety issues yes, there is an officer that has to provide approval.

Kapuscinski: My experience is that these IBCs work very well for having a sense of the level of confinement necessary for research with transgenic organisms. I'm wondering if you need a federal agency to play a major role in regulating that.

Wright: I would agree that there doesn't need to be federal regulation of this. Notification yes, but regulation no. It will chew up a lot of money if you need federal regulation every time you use a GM zebrafish. Risk is present with fish that could escape and survive (i.e., salmon, trout, etc…) and therefore there should be some involvement of federal level agencies in that decision making. I agree that safety is perfectly handled at the university institutional level.

Benfey: I know about the evaluation done in the US for zebra fish. They couldn't find populations even though thousands have been flushed down the toilet for 50 years or so. There are also feral goldfish in New Brunswick.

Fletcher: Is DFO involved at all with university research?

Tsang: We do have draft research policy on research and development which advises R&D of GM fish to be conducted in secure and contained facility.

Devlin: The CCAC has a draft policy, and 100% containment is also a recommendation. The main way in which transgenic fish are currently regulated is through CEPA, but the movement of fish is also regulated by the Fisheries Act.

Wright: [Tells story of DFO involvement in the transfer of Red tilapia to his Dalhousie lab]. (Red tilapia example from his paper as example of DFO involvement).

Devlin: These problems are probably due to fish transfer issues rather than the fact that they were transgenic (This mentioned in response to Jim's story about DFO's involvement with red tilapia).

Bughio: CCAC is a private organization and has nothing to do with government. The CEPA is the authority on GM organisms if they are going to be imported or manufactured or released inside Canada. Environment Canada must be notified prior to import or manufacture of new substances into Canada.

Wright: These transgenic fish are not to be released; the CEPA has a clause that prevents them from being released.

Bughio: Glowfish are not regulated in the US, except in California. The exporters and importers of the Glofish assumed that if the US does not regulate them, then neither will Canada. But this is not the case. All new substances are regulated under CEPA. Importers, exporters, or manufacturers of new substances must notify Environment Canada prior to import or manufacture of their new substances into Canada. According to Section 29.16 of New Substances Notification Regulations of CEPA, new organisms are not subject to CEPA regulations if they are research and development substances that are manufactured or imported in Canada such that there is no release of the living organism, its genetic material, or any material from the organism involved in toxicity, into the environment But if this research and development organism, its genetic material, or any material from the organism is released intentionally or accidentally released into the environment, then you have to notify Environment Canada.

Wright: Nobody makes transgenic mice to release them into the environment. The point being, that if there is no risk of release or no risk of establishment in

local environments then we shouldn't have to jump through the normal regulatory hoops utilized when regulating animals for food consumption.

Bughio: Provided clarification / information on: What is CCAC; Involvement of CCAC in the regulation of transgenic aquatic organisms in Canada; Involvement of CCAC in the evaluation of transgenic aquatic organisms in Canada; Responsibilities of Environment Canada/Health Canada-HECS-Biotech Section in the regulation of transgenic aquatic organisms; Current regulation of transgenic aquatic organisms in Canada; CEPA, NSNR, Section 29.16 - CEPA's regulatory scope and the responsibility of the researcher if the animal or products of the transgenic animals are to be intentionally or accidentally released into the environment); Current status of DFO's Draft Regulations on transgenic fish.

VARIABLES INFLUENCING RISK ASSESSMENT DATA DERIVED FROM LABORATORY-CONTAINED GH TRANSGENIC COHO SALMON

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Abstract

Transgenic fish research has been underway for nearly 20 years, during which time more than 30 species have been genetically engineered for traits including growth, reproduction, disease resistance, and metabolism. The application of transgenic technology will require an understanding of potential ecological risks which could occur in the event of an accidental release of GM fish into nature. Three approaches are being used for risk assessments: 1) genetic, physiological and behavioural assessments to identify effects of transgenesis, 2) development of lab facilities that can mimic nature, and 3) use of surrogate models (i.e., implantation of slow-release GH protein formulation, or domesticated strains with similar phenotypes) to assess fitness directly in nature. Assessment of potential ecological risk in laboratory facilities is complicated by genotype X environment (G x E) interactions which arise in transgenic fish reared in a culture environment. For species such as salmon which are derived from large complex habitats, rearing in culture environments can have very strong effects on phenotype, and for transgenic strains which must be reared in confinement, we do not know the magnitude of these G x E effects. Background genetics effects could also play a significant long-term role in altering transgenic fish phenotypes in nature, arising from the selection of genetic variability within populations to enhance the fitness of the transgenic individuals. These uncertainties in risk assessments make desirable alternatives such as reproductive containment through sterilization by triploidy which are currently very effective in the laboratory, but require enhancement to ensure they are reliable at production-level scales.

Résumé

Des recherches sur les poissons transgéniques sont menées depuis près de 20 ans, période au cours de laquelle plus de 30 espèces ont été modifiées génétiquement afin d'améliorer certaines de leurs caractéristiques, comme leur croissance, leur reproduction, leur résistance aux maladies et leur métabolisme. L'application de la technologie de la transgénèse nécessitera une connaissance des risques écologiques qui pourraient se présenter si des poissons génétiquement modifiés étaient libérés accidentellement dans la nature. Trois approches sont utilisées pour évaluer les risques : 1) des évaluations comportementales, physiologiques et génétiques afin de déterminer les effets de la transgénèse; 2) la construction de laboratoires dans lesquels il est possible de simuler les conditions naturelles; 3) l'utilisation de modèles de substitution (c.-à-d. l'implantation

d'une formulation à libération lente de l'hormone de croissance ou des souches aux phénotypes semblables utilisées en aquaculture) afin d'évaluer la valeur adaptative directement dans la nature. L'évaluation du risque écologique possible en laboratoire est compliquée par les interactions entre des génotypes et l'environnement (G x E) qui surviennent chez les poissons transgéniques élevés dans un milieu de culture. Pour les espèces qui proviennent de grands habitats complexes, comme le saumon, l'élevage dans un milieu de culture peut avoir des effets très importants sur le phénotype. En qui a trait aux souches transgéniques qui doivent être élevées en milieu fermé, nous ne connaissons pas l'ampleur des interactions G x E. Le génotype pourrait également jouer un rôle important à long terme dans la modification des phénotypes des poissons transgéniques dans la nature, à la suite de la sélection de variations génétiques au sein de populations dans le but d'accroître la valeur adaptative des organismes transgéniques. Ces incertitudes dans le cadre des évaluations des risques rendent attrayantes des solutions de rechange, comme le confinement sur le plan de la reproduction par le biais de la stérilisation par triploïdie, qui sont présentement très efficaces en laboratoire, mais qui requièrent des améliorations afin d'assurer leur fiabilité dans le contexte de la production.

Introduction

Genetically engineered species of fish have now been developed for basic research, for medical models, and for enhancing production efficiency of aquaculture (e.g. Figure 1). While very significant potential benefits have been achieved using this technology, at this time, genetically-engineered strains of finfish are not yet known to be used in commercial in aquaculture. In Canada, authority for regulating environmental effects of transgenic fish falls under the *Canadian Environmental Protection Act 1999* as products of biotechnology, and under the federal *Fisheries Act* as substances which are potentially deleterious to fish bearing waters. Fisheries and Oceans Canada has undertaken a noncommercial research program on transgenic salmonids for the past decade with the objectives of 1) developing model transgenic fish strains to provide data regarding benefits and risks of transgenesis, 2) identifying variables and limitations in risk assessments, and 3) improving our basic understanding of genetic and physiological controls of phenotype with an emphasis on growth. The information below briefly summarizes our findings derived from research with transgenic salmonids containing growth hormone gene constructs, as well as some concepts and limitations experienced in attempting to derive risk assessment data.

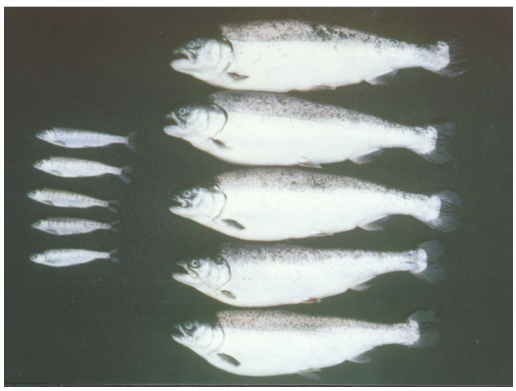

Figure 1. Nontransgenic and GH transgenic coho salmon at one year of age.

Approaches to environmental risk assessment

The potential for transgenic fish to accidentally enter natural ecosystems makes prudent an *a priori* assessment of their potential to establish in nature and their potential to cause ecological damage (Kapuscinski and Hallerman 1990). Critical factors for risk assessment of transgenic fish include 1) the probability and magnitude (frequency and numbers of individuals) of escapes from culture facilities into nature, 2) direct impacts of escaped fish (e.g. competition for resources, or resource degradation), and 3) sustained multigenerational impacts of escaped fish. The persistence of transgenic fishes in ecosystems will depend on their overall fitness relative to wild fishes. Fitness is comprised of two main components: the probability of surviving from zygote to sexual maturation combined with the magnitude of reproductive output (e.g., probability of successfully mating and consequent numbers of viable and fertile offspring produced). Many subcomponents of survival and reproductive success contribute to overall fitness. Modeling shows that small differences in fitness over time can result in potential impacts on fish populations. Indeed, certain combinations of fitness states are suspected to be able to result in serious population effects including extinctions (Muir and Howard 1999; Muir and Howard 2002).

Fitness data for genetically modified salmon derived from nature would provide the best estimates for risk assessments, allowing all of the myriad of complex variables to interact and culminate in a final assessment of fitness. However, for fish, this is clearly not feasible due both to social opposition as well as legitimate scientific concern regarding potential environmental consequences. Thus, risk assessments with transgenic salmon have been to date performed in contained facilities, in contrast to the approach taken for many plant GMOs where fitness data can be directly acquired from field trials conducted in nature.

With the need for containment, how can empirical risk assessment data be acquired for a species such as salmon, which normally reside in a large and complex environment? And, equally important, what limitations does the laboratory approach present regarding the reliability and thus utility of resulting risk assessment data? Three basic approaches are currently being explored:

1. Examination of individual genetic, physiological, and behavioral characteristics of transgenic and control animals under controlled laboratory conditions (see below).
2. Establishment of artificial laboratory facilities that mimic natural environments (e.g., Sundstrom et al. 2004).
3. Development of living nontransgenic models which can be released into nature (e.g., to mimic GH transgenic fish, the use of fast-growing domesticated strains, or GH protein implanted fish (Johnsson et al. 1999)).

Physiological and behavioral phenotypes of GH transgenic coho salmon and trout

Overexpression of GH in stable lines of transgenic salmon and trout (Du et al. 1992; Devlin et al. 1994; Devlin et al. 1995; Pitkaenen et al. 1999; Devlin et al. 2001; Devlin et al. 2004) has been observed to affect growth at many life history stages. This rapid acceleration of development allows transgenic fish to emerge earlier from natal nests, precociously enter the marine environment (smolt) at the end of their first summer of life, and reach sexual maturity at a younger age (2 vs 3-4 years, but at normal adult body size).

In addition to growth effects, many other pleiotropic effects of GH overexpression have been detected including physiological effects on muscle structure, metabolic enzyme levels, maximum swimming speed, swimming economy, aerobic metabolic rate, eye development, disease resistance and stress response, intestinal structure, feed conversion efficiency, and nutrition metabolism (Farrell et al. 1997; Cook et al. 2000; Cook et al. 2000; Hill et al. 2000; Stevens and Devlin 2000; Blier et al. 2002; Jhingan et al. 2003; Lee et al. 2003; Leggatt et al. 2003). Reproductive biology may also be affected: females have higher fecundity but produce smaller eggs than wild females, whereas males seem less influenced and produce normal sperm quantity with equivalent fertilization ability (Bessey et al. 2004). However, spawning success of transgenic salmon has been found to be significantly reduced relative to wild salmon (Bessey et al. 2004).

GH transgenic salmon possess dramatically-enhanced feeding behaviour with improved ability to compete for available food resources (Abrahams and Sutterlin 1999; Devlin et al. 1999; Sundstrom et al. 2004). Under high food availability, growth of nontransgenic salmon is not affected by the presence of transgenic cohorts in the same environment, whereas at normal or low food availability, transgenic fish are able to outcompete and suppress the growth of nontransgenic animals (Devlin et al. 2004). At first consideration, enhanced ability to acquire available food resources might be considered a fitness advantage for GH transgenic salmon. However, to maximize fitness, salmon in nature make a tradeoff between leaving cover to forage for prey items versus the risk of exposing themselves to predation, whereas GH transgenic salmon have been shifted behaviourally such that their desire to forage for food is dramatically enhanced. Recently, experiments in semi-natural environments containing predators have shown that GH transgenic fish suffer greater mortality than nontransgenic cohorts (Sundstrom et al. 2004). Such effects on early fry survival, if they occurred in nature, would strongly reduce the fitness of transgenic animals relative to wild salmon.

Major factors influencing reliability of risk assessment data

The above experiments assessing phenotypes of GH transgenic salmonids have revealed a complex array of pleiotropic effects. Many factors are anticipated to influence several fitness components, and artifacts of the experimental facilities are playing a significant

role. Currently, there are several major limitations to providing reliable risk assessment data on potential ecological effects of GH transgenic salmon.

1. A great deal of empirical information is accumulating from individual laboratory experiments on physiological and behavioral differences which exist between transgenic and nontransgenic salmon, and this has provided an efficient way to identify consequences of transgenesis. However, in most cases, it is very difficult to fully determine how such differences might influence fitness in nature without applying very large assumptions (for example, swimming performance differences may be detected in transgenic salmon, but to what degree would this affect ability to avoid predators, catch prey, or successfully migrate up spawning rivers?) Further, the complexity of interacting effects arising from the same phenotypic state (e.g., enhanced feeding behaviour provides a fitness advantage for food acquisition, but a fitness disadvantage for predator avoidance) indicates that application of specific observations is difficult.

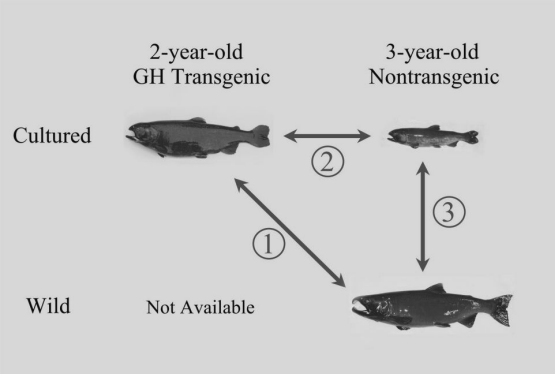

Figure 2. Mature coho salmon derived from different rearing environments

2. Data generated from laboratories are inherently subject to two major effects; first, the laboratory environment can only a partially imitate nature, and thus, the exact conditions of an experiment may have significant effects on the data acquired. More seriously, specific conditions may influence wild and transgenic genotypes in different ways (genotype x environment interactions) (Kareiva et al. 1996; Devlin et al. 2004). Thus, risk assessment data should be collected from a broad range of experimental conditions, and ideally from environments which mimic nature as closely as possible. For example, while strong population extinction effects have been observed in simple environments containing GH transgenic salmon, it is not certain that such effects would occur in nature where populations are much larger and the opportunity for emigration and hiding behaviour exists (Devlin et al. 2004). A second major factor influencing utility of laboratory risk-assessment data arises because the test transgenic organisms have (necessarily) been raised within the laboratory facilities. For salmon, the culture environment itself can have very significant effects on many phenotypes, including growth. For example, growth of wild-strain coho salmon in laboratory-scale aquarium facilities is consistently suppressed below that seen for wild fish in natural environments (Bessey et al. 2004; Devlin et al. 2004), whereas GH transgenesis largely suppresses this effect and results in enhanced intake of food and growth. Comparison of transgenic fish with wild salmon (both of which attain a similar body size at maturity, but the latter are one year older) leaves uncertain to what degree rearing environment versus transgene

action plays a role in development of their phenotypes (Figure 2, arrow 1). Experimentation with cultured nontransgenic wild-strain coho salmon (which are one to two years older yet have much smaller body sizes than transgenic salmon) results in comparisons with phenotypically abnormal (small body size) fish (Figure 2, arrow 2). Without fully understanding the effects of environmental control of specific phenotypes within genotypes (Figure 2, arrow 3), and knowledge of differences in these effects between genotypes, we have an uncertain basis from which to extrapolate the phenotype of transgenic salmon which have lived their entire lives in the wild. Yet, it is this latter unavailable phenotype that we need to know for accurate risk assessments. Because of this limitation, application of data derived from comparisons of wild (nontransgenic) salmon and cultured transgenic salmon should be limited to that which might occur at first contact if escaped aquacultured transgenic fish entered wild populations (Bessey et al. 2004).

3. The expression of a transgene appears to be dependant on background genetic effects. For salmonids, wild strains (which have genetic backgrounds which cause slow growth) respond strongly to GH transgenes, whereas a domesticated strain (which has a genetic background selected for rapid growth) shows a reduced response to GH transgenesis (Devlin 2001). Such background genetic effects could result in very different selection consequences for a transgene among different strains in nature, thus amplifying the complexity of conducting reliable risk assessments since determinations of phenotype and fitness are then moving evolutionary targets. Modeling studies (unpublished) have indicated that very small shifts in selection intensity acting on a transgenic phenotype can have important implications for long-term effects on populations.

Containment approaches

The uncertainties described above in assessing environmental risk of fertile genetically distinct fish suggest that physical and biological containment may be the best approach to minimizing potential consequences (Devlin and Donaldson 1992). The first line of defense against potential environmental impacts of GM fish is to minimize the probability they will enter nature by employing highly-secure physical containment systems. Since open-ocean net pens are known to fail with low but virtually certain probability, the use of land-based facilities is clearly preferred. Unfortunately, land-based culture for many aquacultured species is not economically feasible due to the prohibitive cost of infrastructure. Consequently, biocontainment approaches have also been considered, including methods for sterilization and reduction of viability.

Currently, a technology considered to be highly effective and sufficiently practical for large-scale application is sterilization by induced triploidy in monosex female populations. Triploidy can easily be induced in salmonids by simple application of a brief, high-pressure shock shortly after fertilization (Benfey 1999). This technology can be highly effective, resulting in 100% triploidy in some cases. In large-scale trials involving transgenic coho salmon, we have also found high levels of induced sterilization

are possible, with an overall efficacy averaging 99.8% (unpublished observations). Such levels of sterilization would provide a high level of biosecurity, and if coupled with a transgenic strain with reduced fitness would provide significant protection for the environment. Reductions of fertile transgenic salmon in populations to low levels by triploidy induction also allows stochastic processes (prevalent in salmon life history due to high fecundity and mortality) to influence the probability a transgenic animal will survive to maturity.

From a risk assessment perspective, it is important to note that, in rare cases, escapes of fish from aquaculture facilities can consist of very large numbers of individuals (e.g., 50,000). In such cases, triploidy failure rates as described above could result in the release of some 100 diploid fertile animals into nature. Thus, while a significant approach for reducing risk, pressure-induced triploidy is, as yet, not a completely reliable approach for biocontainment. The extent to which fertile diploids in otherwise triploid groups would result in ecosystem effects in nature would depend on their fitness as discussed above, and while high level of sterilization may delay effects, they would in many cases not be prevented if escapes of transgenic fish occurred on a regular basis. From the perspective of suitability for aquaculture, triploid strains of GH transgenic fish have been found to have reduced growth rates relative to diploids, although still significantly greater than either diploid or triploid nontransgenic fish (Razak et al. 1999; Devlin et al. 2004).

To further improve biocontainment, other approaches including transgenesis are being explored to enhance levels of sterilization and/or viability reduction. Such approaches will themselves need to be subjected to independent risk assessments. For those species with a stable genetically-based sex determination system, and the potential to conduct aquaculture in areas where no conspecifics exist, the use of alternate biotechnology approaches such as monosex female culture may provide a very simple yet highly-effective approach to biocontainment (Devlin and Donaldson 1992).

Acknowledgements

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Participant Discussions

Maclean: My feeling is that we should make a distinction between the effects of a particular transgenic strain and the effects that growth enhancement might have outside of transgenesis. Might the rapidly increased growth actually be responsible for phenotypic changes? (**Statement)**

Devlin: We are currently examining that issue in the lab, comparing domesticated strains vs. transgenic strains to determine what is common to growth processes in domestics and transgenics.

Kapuscinski: I get the sense that a lot of this is still private but not published yet. Am I recalling correctly that wild transgenic trout, in one of your papers, were much larger at sexual maturity than the non transgenic trout?

Devlin: The information is not private, but quite a bit remains unpublished at this point. Yes, trout do mature at a larger size, data which were published a

couple of years ago. We also have data to suggest their reproductive development is strongly impaired in transgenic fish when GH production is too high.

Kapuscinski: I raised the question, in terms of interest in fitness and transgene fate, for the following reasons. What is the size of the fish when they are sexually mature, that is, what size are they at sexual maturity and how fecund are they? How would these traits affect the spread of a gene or transgene?

Devlin: Broodstock sterilized by triploidy induction don't die after spawning and may continue to grow. It's when sexual reproduction occurs that they stop growing and die. In trout they don't die, and in some strains, transgenesis impairs reproduction. For example, in GH transgenic trout, growth continues after sexual maturity and they become larger than normal. Currently, lab culture effects are preventing us from obtaining reliable reproductive fitness data, and these are probably more powerful effects on reproductive fitness than is fish size.

APPLICATION OF MODELING TO RISK ASSESSMENT OF GM FISH: THE NET FITNESS APPROACH

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Abstract

Containing possible risk from GE fish can be divided into mechanical isolation and biological. The most common method of biosecurity is sterilization. A second method is through assessment of transgene effects on fitness. If fitness is compromised, then natural selection will eventually eliminate the transgene. The net fitness approach is designed to measure life history characteristics and address that latter issue Predicting the outcome of natural selection is a two step process involving (1) estimation of net fitness components for alternative genotypes, and (2) incorporation of parameters into a model that predicts change in gene frequency and population size. The critical fitness components have been reduced to: juvenile viability, age to sexual maturity, mating success, female fecundity, male fertility, and adult viability. The specific mechanisms (e.g., physiological, behavioral, immunological processes) that underlie an observed difference in any fitness component need not be identified to assess risk, thus saving considerable time and expense. The approach then combines estimates of the net fitness parameters into a mathematical model to determine the fate of the transgene in the affected wild population. This estimate would help decision makers determine potential for risk and if additional measures should be used to contain the fish. Alternatively in the design stage of GM organism, the method can be used to manage risks by answering the question of "what aspects of the life history of the organism result in spread of the transgene." Efforts may be directed to change or mitigate those attributes of the transgene. Use of this model will allow regulators to define a consistent set of criteria for animals to be evaluated as part of a standardized risk assessment program.

Résumé

Les mesures de confinement visant à limiter les risques que pourraient présenter les poissons génétiquement modifiés peuvent être classées en deux catégories : les mesures biologiques et les mesures d'isolement par des moyens mécaniques. La méthode la plus fréquente pour assurer la biosécurité est la stérilisation. Une deuxième méthode consiste à évaluer les effets du transgène sur la valeur adaptative. Si cette valeur est compromise, la sélection naturelle finira par éliminer le transgène. L'approche axée sur la valeur adaptative nette est conçue de façon à mesurer les caractéristiques du cycle vital et à régler cette dernière question. La prévision du résultat de la sélection naturelle nécessite un processus à deux étapes : (1) l'estimation des caractéristiques de valeur adaptative

nette pour d'autres génotypes; (2) l'intégration des paramètres dans un modèle de prévision des changements sur le plan de la fréquence de gènes et de la taille des populations. Les caractéristiques de valeur adaptative essentielles ont été limitées à la viabilité des juvéniles, à l'âge de la maturité sexuelle, au succès de reproduction, à la fécondité des femelles, à la fertilité des mâles et à la viabilité des adultes. Les mécanismes précis (p. ex. les processus immunologiques, comportementaux et physiologiques) qui sous-tendent une différence observée sur le plan de toute caractéristique de valeur adaptative n'ont pas besoin d'être identifiés pour évaluer les risques, ce qui permet d'économiser beaucoup de temps et d'argent. L'approche comprend ensuite la combinaison des estimations des caractéristiques de la valeur adaptative nette dans un modèle mathématique afin de déterminer le devenir du transgène dans la population sauvage concernée. Cette estimation aiderait les décideurs à déterminer le potentiel de risque et à établir si des mesures supplémentaires doivent être prises pour confiner le poisson. Par ailleurs, dans le cadre du stade de conception de l'organisme génétiquement modifié, la méthode peut être utilisée pour gérer les risques en répondant à la question suivante : « Quels aspects du cycle vital de l'organisme entraînent la propagation du transgène? ». Des travaux pourraient être axés sur la modification ou l'atténuation de ces caractéristiques du transgène. L'utilisation de ce modèle permettra aux responsables de la réglementation de définir un ensemble cohérent de critères pour les animaux aux fins d'évaluation dans le cadre d'un programme normalisé d'évaluation des risques.

Introduction

Transgenic technology is rapidly developing as techniques for transforming fish and other organisms (Muir and Hostetler 2001). Current limitations of this technology lie in construct development. These limitations include: stability, position independence, regulation, tissue for expression, and equally important, which genes to insert. Our information base on all of these aspects is improving rapidly as genome characterizations of humans and other organisms become available. Soon gene constructs will be possible for nearly any use and species. However, even if the biological limitations of transgenic technology are overcome, the problem of public acceptance persists.

Consumers and environmentalists remain wary of the safety of biotechnology in agriculture. Research is needed to increase consumer confidence and to alleviate this concern. The first step in this process is to develop a risk assessment methodology that is agreed upon by scientists. If scientists cannot agree on a unified methodology for testing, the public will reject transgenic technology on the basis of uncertainty. This goal can best be accomplished using a thorough, unbiased examination of risks and hazards associated with agricultural biotechnology. Muir and Howard (1999, 2001, 2002a,b, 2004) examined possible hazards of transgenic fish and developed a method to estimate environmental risk of genetically modified (GM) organisms. Their method was based on a combination of standard risk assessment methodologies and biological modeling. In the

following sections, these methods, limitations, and associations with classical risk assessment methodology will be discussed.

Understanding how the net fitness method integrates with risk assessment

Our approach uses definitions of risk and its components in a manner consistent with the NRC (1983) Red Book (and its quantitative application in a context consistent with ecological risk assessment). Thus, **harm** (an undesirable outcome) is considered from the standpoint of **hazard** (harm as affected by the potential environmental stressor) and **exposure** (the environmental occurrence of the stressor). **Risk** is the likelihood of harm resulting from exposure to the hazard under environmentally relevant conditions. Thus, in the case of a transgene as stressor, the risk is a joint probability of harm given the transgene spreads, P(H/E), and the probability of the transgene spreading, P(E):

Risk = P(H/E) x P(E)

The probability of exposure is the product of at least two parts, one conditional on the other. The first part is species dependent and relates to the ability of the organism to escape, disperse, and become feral (NRC, 2002). The second part is the ability of the transgene to spread given the gene has been introduced into the population by an escaped animal.

P(E)= P(Escape) x P(Transgene Spread/Escape)

Although risk can be defined in precise mathematical formula, our ability to quantify the various parts can be formidable or even impossible. Nevertheless, such formulas allow us to understand the flow of risk and interrelationship between aspects. Because risk results from a chain of events (escape, followed by spread, followed by harm), the analogy of a chain being only as strong as its weakest link can be utilized. As such, it is not necessary to quantify all aspects of risk if the probabilities of any of the links can be shown to be close to zero. The weakest link is the upper limit of risk.

The first link, probability of harm given exposure (sometimes called the "*so what*" question), is the most difficult to determine. A major limitation of environmental risk assessment for any stressor is the near infinite number of biotic interactions possible in and between ecosystems, thus it is not realistic to anticipate all possible harms from exposure to the stressor. Even for those harms that can be anticipated, such as species displacement or extinction, the probability of such an event resulting cannot be predicted with our current state of the science even with fore knowledge that invasion by a given species or GM organism will occur. Thus it is not easy to dismiss this link as being unlikely.

The second link, the probability of escape, spread, and become feral is species dependent and was considered by the National Academy of Science, National Research Council on

science-based concerns of animal biotechnology (NRC, 2002). They considered aquatic organisms and insects as having the highest level. The reasons for these concerns were precisely because aquatic organisms and insects could escape easily, especially in juvenile forms; neither are highly domesticated, and both have wild counterparts to mate with. Thus they will easily become feral and our waterways are interconnected so spread is likely. Thus this link is known to be strong and cannot be dismissed for aquatic organisms.

The last link, which the transgene spreads given escape, depends on the forces of natural selection, the same factors which are universal to all organisms. Quantifying the ability of the transgene to spread can be approached directly, and is amenable to universal rules of population genetics dependent on natural selection; it, therefore, represents the part of the risk formulation that population genetics can address.

Methods of containment of GM organisms were recently addressed by the NRC (2004). However, it should be recognized that all methods of containment can be put in terms of exposure in these equations. Physical containment is directed at the second link that of eliminating escapes. Biological containment, usually sterility methods, is directed at the third link, the ability of the transgene to spread given that escape has occurred. The methods of Muir and Howard (2001, 2002a,b, 2004) also concentrate on this last link and make the case that if the transgene will not spread, then risk approaches zero as a consequence. In essence, the transgene is biologically constrained even though the parents are not sterile.

Models to predict transgene spread

Predicting the outcome of natural selection is a two step process involving (1) estimation of net fitness components for alternative genotypes, and (2) incorporation of parameters into a model that predicts change in gene frequency and population size. Prout (1971) originally described the relationship of net fitness components and population prediction, stressing a general approach with a small set of components, encompassing the entire life cycle, and amenable to experimental evaluation. Net fitness components and life history characteristics are synonymous terms. As reformulated and expanded by Muir and Howard (2001), six net fitness components allow for the quantitative description of the outcome of natural selection: juvenile viability, age to sexual maturity, mating success, female fecundity, male fertility, and adult viability (Figures 1-5). The specific mechanisms (e.g., physiological, behavioral, immunological processes) that underlie an observed difference in any fitness component need not be identified to assess risk, thus saving considerable time and expense. The approach then combines estimates of the net fitness parameters into a mathematical model to determine the fate of the transgene in the affected wild population (given by Muir and Howard 2001). To make these methods more accessible and understandable, they have been posted and discussed with examples at two web sites (Muir, 2001a,b, 2002a,b).

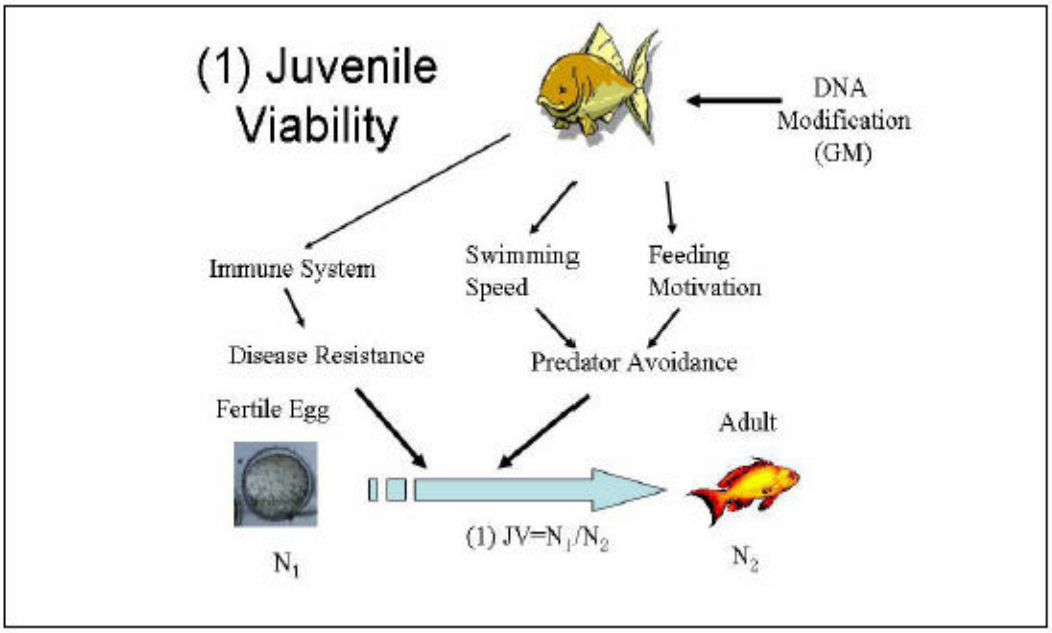

Figure 1. All impacts of genetic modification on physiology and resulting survival difference can be measured simply as the proportion surviving to sexual maturity.

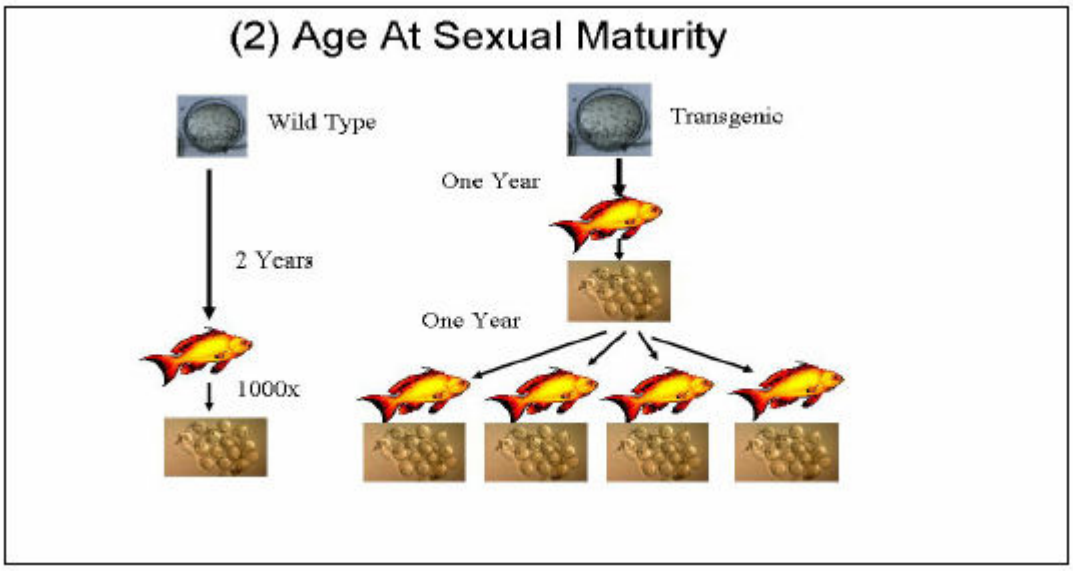

Figure 2. Age at sexual maturity is a critical factor in population expansion as it determines the number of generations that can occur per unit of time.

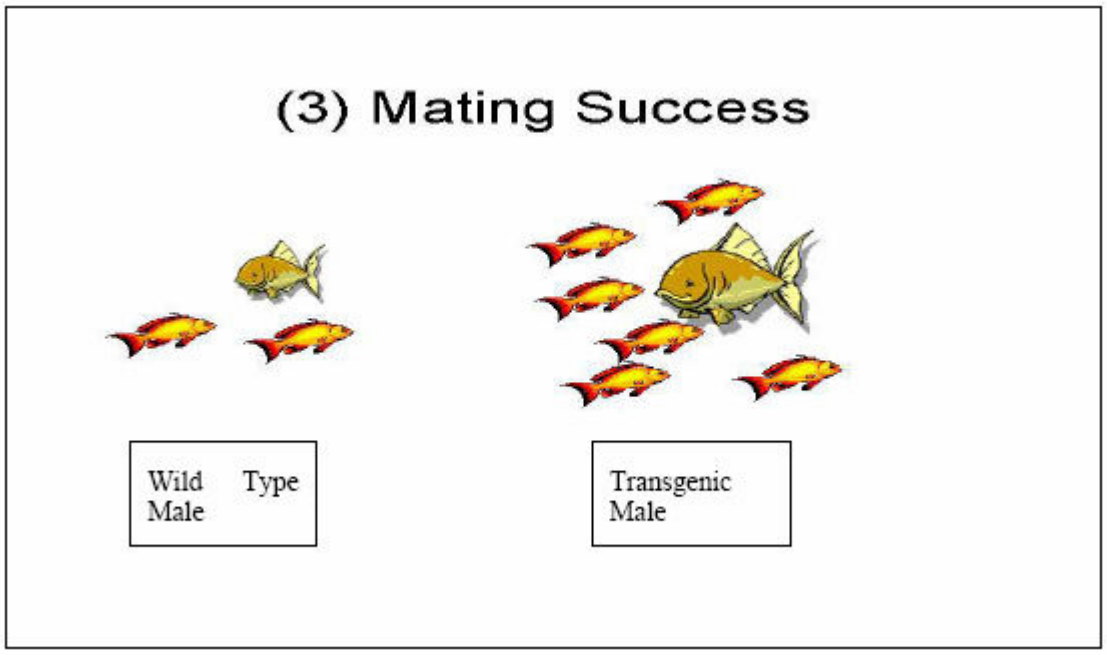

Figure 3. Differential mating success (sexual selection) is one of the strongest forces of evolution in natural populations, often stronger than viability selection.

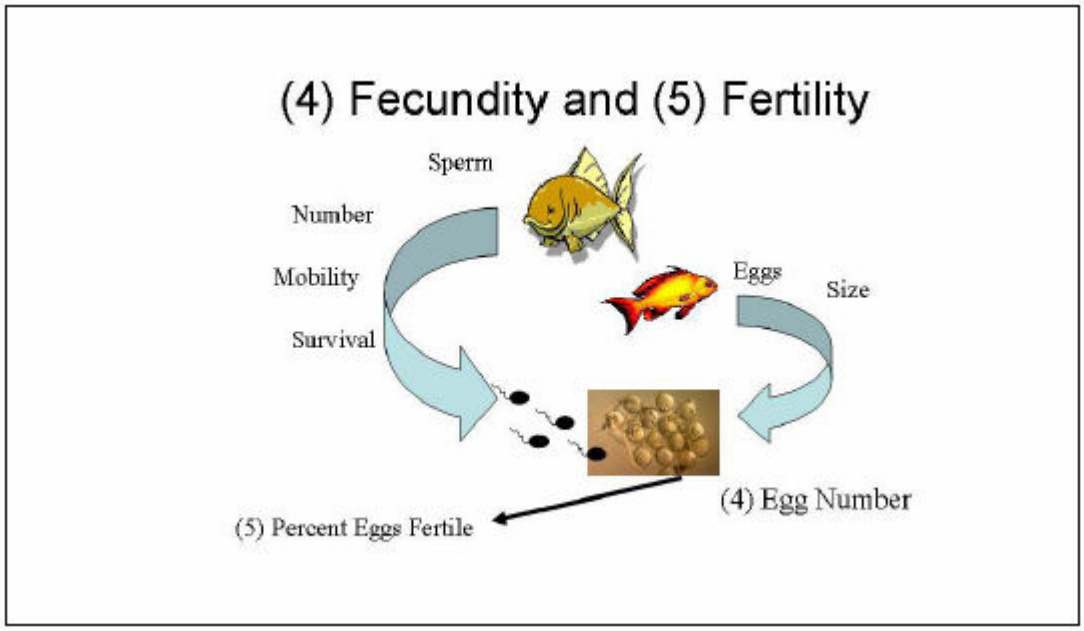

Figure 4. Relative fecundity (egg number) and fertility determine the number of offspring produced per mating pair.

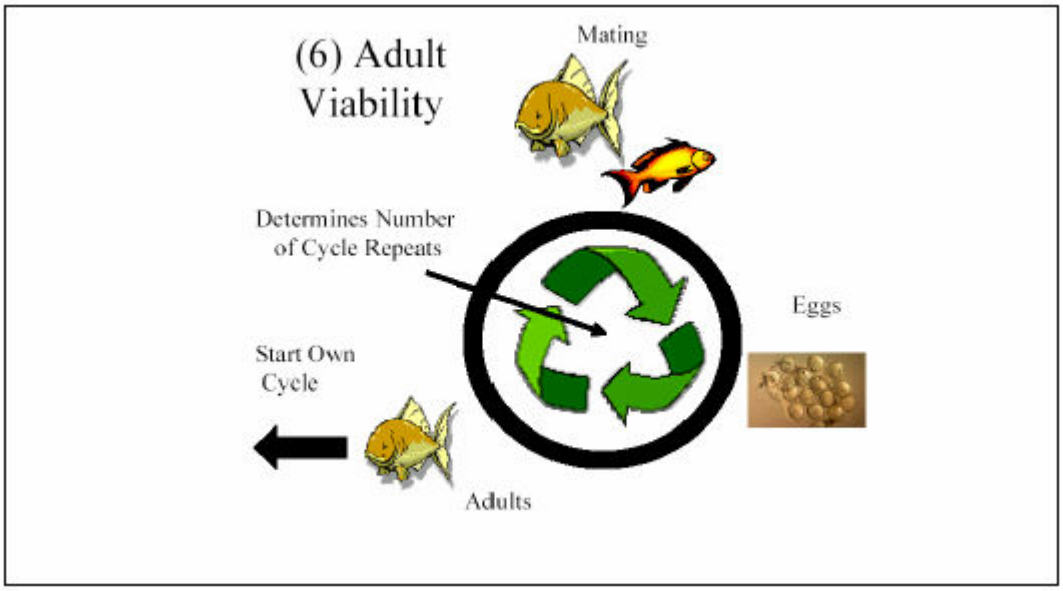

Figure 5. Adult viability determines the number of times a mating pair can repeat the mating process.

Utility

If the probability of transgene spread is high, as determined by our models, then alternative containment efforts can be concentrated on those cases where potential harms are great. For example, if sterility is used for containment, should every fish be tested for sterility? If only a sample is used for quality control, how many should be sampled? The 2004 NRC report on Biological Confinement of Genetically Engineered Organisms notes that "The net fitness method (Muir and Howard 2001, 2002) provides a means to estimate—in a secure setting—the probability of severity of the harmful consequence from such transgene spread. This estimate would help decision makers determine whether to screen all or only a sub-sample of each production lot. If they choose sub-sampling, this estimate would help determine the appropriate sample size as a function of the predicted severity of harm, the probability of harm given an escape of fertile salmon has occurred, and the probability of escape of fertile fish." (NRC 2004).

Alternatively in the design stage of GM organisms, the method can be used to manage risks by answering the question of "what aspects of the life history of the organism result in spread of the transgene?" Efforts may be directed to change or mitigate those attributes of the transgene.

Limitations

Our approach cannot eliminate the need to assess for rare events with catastrophic consequences; but given that these consequences cannot be demonstrated on regulatory timescales, the emphasis must remain on cautious prediction of transgene spread as a necessary first step in risk assessment.

Limitations can be grouped into two categories: those related to the model and those related to the estimates used to parameterize the model.

No model can be perfect or omnipotent, we strongly believe our method encapsulates the most important components of an organism's life cycle. Because of the interest in these methods to manage environmental risk, two workshops were convened by Information Systems for Biotechnology. The workshops consisted of a diverse panel of regulators, population geneticists, evolutionary biologists, behavioural ecologists, modelers representing taxonomic expertise on fish, insects, and plants to discuss limitations and suggest improvements. The first workshop was in summer 2002 and addressed issues related to animals, particularly fish (Hallerman 2002).

The second workshop was convened in summer 2003 to address issues related to plants as well as some of the concerns identified by the first workshop (ISB, 2004). The participants of the ISB Workshops recognized the model's basis in population genetics and evolutionary theory but also recommended a number of ways in which the net fitness model could be improved or extended. In its current form, the model is deterministic, i.e., the input parameters absolutely determine its predictions. The workshops made two major recommendations to expand the model:

1) Stochasticity should be incorporated to include effects of random events that are likely in finite populations, such as failure to find a mate, or gene swamping due to large number of domestic fish escaping into a relatively small natural gene pool.

2) Uncertainty needs to be incorporated into the model. There is uncertainty in both the fitness component estimates, and the outcome of natural selection. Both of these uncertainties need to be reflected in model predictions; i.e., a credibility interval is needed for the probability of a particular outcome. A suggestion was made to use Bayesian methods because they incorporate prior information and have several other desirable characteristics).

Another problem with estimating fitness components is the genetic background. The degree to which genetic background effects are linked to the transgene will be of consequence to the evolutionary fate of the transgene in the wild. If the transgene is inserted adjacent to polymorphic genes which impact fitness in some way, the joint effects of these genes must be considered because, although linkage effects dissipate in the long term as recombination events occur, in the short term linkage disequilibrium will

be high because of hybridization upon outcrossing. Because transgenes are usually inserted into domesticated animals, which have been selected for increased productivity but are poorly adapted to natural environments, the transgene will most likely be linked with maladaptive alleles under natural conditions. Thus linkage disequilibrium presents a challenge because use of the net fitness approach would underestimate the true benefit of the transgene in natural environments. Such issues can be addressed by crossing the GM fish into a wild background and randomly mating for a few generations to dissipate linkage disequilibrium before fitness components are estimated.

Conclusion

Use of this model will allow regulators to define a consistent set of criteria for animals to be evaluated as part of a standardized risk assessment program. Without a consistent and agreed upon set of criteria for evaluation of environmental risk, it is not possible for either developers or regulators to determine when a particular genetic modification presents an environmental risk.

The public also must be assured by scientists and regulators that if a GM animal is released, it does not present a significant risk. The alternative is to work with contained or sterilized GM animals.

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Participant Discussions

Dunham: I fully understand that it's impossible to measure everything. I would contend that juvenile viability is a key component in this model. In nature there are two primary reasons juveniles die: they starve, or they are eaten by someone else. Even though you can't measure a hundred different things, it's important to set up experiments to measure those components. Food type is also going to affect viability and competition. In nature they

are not going to be competing for pellets so for this data to be something we can make strong predictions with, much of this data must be acquired using natural food sources and natural predators. On your conjecture that no risk in lab equals no risk in nature model, this is perhaps not necessarily true because transgenic animals could have increased predator avoidance, therefore we could accidentally release an animal to the environment that might have increased avoidance. We are in danger of making the wrong conclusions.

Muir: If this is true, then we can only rely upon proper containment procedures. I've concluded that the more realistic your scenarios, the more realistic your predictions. The experiments should be done using optimum conditions for both organisms as well. If the differences are the same using both methods (optimum conditions/wild type conditions - i.e., decreased fecundity), then the conjecture can stand.

Dunham: My primary point is they are going to be exposed to food and predators.

Unknown: You can argue that those two components should always be part of the fitness approach. The more realistic you make the situation, the more accurate your prediction. I always look at the range in experiments, such as both extremes of food and predators. How robust is this? Is this variable thing always going to be a risk, or is it based on a specific scenario?

Unknown: I would contend that lack of food or abundance should be natural. They are never going to run into a lake with a bunch of pellets. I can see low number of predators vs. high number, or no predators.

Kapuscinski: This is a comment more than a question. A lot of us are talking about the same point: it's extremely important to figure out what range of environmental conditions you measure. I think that what you really have to do is look at the species your dealing with, and what the most limiting biotic and abiotic factors are. Because you can't test all of them, limit which you can figure out and develop a range you can assess for them. There could be a species out there where predatory avoidance is not important but food availability is. We need to hook up with top notch population ecologists and community ecologists. What are the factors and what are the key tests?

Biagi: Is the assumption made that the larger fish is always of greater value for mating success?

Muir: Measure mating success based on data, regardless of it if was large or not. In some fish it's mating display not size that determines mating success.

Donaldson: What about the sneaker phenomenon?

Muir: The model has incorporated this and the larger sneakers have a competitive advantage over the smaller ones.

STATUS OF ENVIRONMENTAL BIOSAFETY SCIENCE ON GENETICALLY ENGINEERED FISH AND POLICY IMPLICATIONS

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Abstract

Biotechnologists are inserting diverse transgenes into finfish, shellfish, and algae for aquaculture, ornamental fish markets, bio-factories, biological control, and contaminant detection. Biosafety science is not well equipped to reach reliable and broadly trusted decisions regarding the environmental safety of aquatic genetically engineered organisms (GEOs). In risk assessment, the science is best developed to identify hazards; lacks a confirmed methodology to estimate exposure to the hazard; and is least developed for assessing risk and severity of harm, given exposure to the hazard. In risk management, discussions have focused on reducing risk via a mix of confinement methods and largely ignored planning for risk monitoring and remedial action. Policies governing aquatic GEOs should stress assessment of uncertainties (given the limits to risk assessment); comparison of risks between using a GEO and alternative approaches; risk analysis by interactive and interdisciplinary teams; and development of clear environmental safety standards. Experience from longer-existing industries and research on public understanding of risk indicate that the best way to reach broad and long-term social acceptance of safety decisions for aquatic GEOs will be to involve scientists in open analysis and potentially affected parties in representative and transparent deliberations to develop safety standards for risk assessment and management.

Résumé

Les biotechnologistes insèrent divers transgènes dans des poissons, des mollusques et des algues aux fins d'aquaculture, de vente sur les marchés de poissons d'ornement, de bioproduction, de lutte biologique et de détection de contaminants. Les responsables de la biosécurité ne sont pas bien équipés sur le plan scientifique pour prendre des décisions fiables et globalement acceptées en ce qui concerne les risques que présentent les organismes aquatiques génétiquement modifiés pour l'environnement. Dans le cadre de l'évaluation des risques, les connaissances scientifiques conviennent davantage à l'identification des dangers (il manque une méthode éprouvée pour estimer l'exposition au danger) et moins à l'évaluation des risques et de la gravité des dommages en fonction de l'exposition aux dangers. Les discussions tenues dans le cadre du processus d'évaluation des risques ont porté sur la réduction des risques par le biais d'une combinaison de méthodes de confinement et d'une planification, en grande partie passée sous silence, de la surveillance des risques et des mesures correctives. Les politiques qui

régissent les organismes aquatiques génétiquement modifiés devraient insister sur l'évaluation des incertitudes (compte tenu des limites de l'évaluation des risques), la comparaison des risques que présentent les organismes génétiquement modifiés et ceux que présentent d'autres approches, l'analyse des risques par des équipes pluridisciplinaires et interactives, et l'élaboration de normes claires en matière de sécurité environnementale. L'expérience d'industries qui existent depuis plus longtemps et une recherche sur la compréhension du risque qu'a le public indiquent que la meilleure façon d'assurer l'acceptation sociale générale et à long terme des décisions en matière de sécurité liées aux organismes aquatiques génétiquement modifiés consiste à faire participer des chercheurs à une analyse ouverte et les parties qui peuvent être touchées par les décisions à des débats transparents et représentatifs dans le but d'élaborer des normes de sécurité aux fins d'évaluation des risques et de gestion.

Introduction

Genetic engineers in the public and private sectors are inserting an increasing diversity of transgenes into an increasing diversity of aquatic organisms (Kapuscinski 2003, NRC 2004). In this paper, 'genetically engineered organism' and 'transgenic organism' (GEO) refer to organisms bearing man-made recombinant DNA genetic constructs (NRC 2002, 2004, Scientists' Working Group on Biosafety 1998, Letourneau and Burrows, 2002). Table 1 summarizes representative examples of the growing diversity of aquatic GEOs that are being engineered for aquaculture to produce human food; as bio-factories to produce pharmaceuticals, industrial chemicals or dietary supplements; for biological control of nuisance invasive species; for recreational markets; in bio-remediation to remove contaminants from water; and as water quality monitors to detect contaminants that damage genes of living organisms. Growth-enhancement for human food production in aquaculture is the most common objective of current efforts but it may not remain so for much longer. Indeed, the first commercially marketed genetically engineered animal in the United States and several other countries was for the ornamental fish aquarium market: the GloFish^{TM}, a transgenic fish with red or green skin color due to skeletal muscle expression of a fluorescent-protein genetic construct (Gong et al. 2003, FDA 2003, Pollack 2004).

Recent trends therefore suggest one should expect future requests for commercial approval of aquatic GEOs to reflect this growing diversity of objectives, species, transgenes, and target traits. The current state of biosafety science and practice for aquatic GEOs is not up to the task of making scientifically reliable and broadly trusted safety decisions about this breadth of transgenic aquatic organisms. As risk assessment research studies understandably focus on the few lines of growth-enhanced transgenic fish approaching or under regulatory review, scientists should also be pro-active in addressing the need to strengthen biosafety scientific methods and empirical data for the coming diversity of aquatic GEOs. Biosafety policy and regulations also need to have adequate scope and breadth to govern this growing diversity aquatic GEOs. In this paper,

the author reviews the status of science needed to inform decisions about the environmental biosafety of transgenic fish; and points out some policy implications of the current status of biosafety science and of lessons from other technology arenas regarding how to gain broad public trust in complex risk decisions.

Table 1. Examples of genetically engineered aquatic organisms under development, (updated from Kapuscinski 2003 and NRC 2004).

Species	Target Engineered Trait [Inserted Transgene]	Proposed Application	Status of Development
Finfish			
Mud Loach	Increased growth rates, improved feed conversion and likely sterility [mud loach growth hormone gene driven by mud loach ß-actin regulatory region] (Nam et al. 2001, Nam et al. 2001a)	Aquaculture for human food	Research
Channel Catfish	Enhanced bacterial resistance [moth peptide antibiotic, cecropin B gene] (Dunham et al. 2002)	Aquaculture for human food	Research
Grass Carp	Increased resistance to grass carp haemorrhage virus [human lactoferrin gene] (Zhong et al. 2002)	Aquaculture for human food	Research
Medaka	Transgenic fish serve as detector of mutagens in environmental pollutants [bacteriophage vector serving as a mutational target]. After exposure to mutagenic agent, vector DNA is removed, inserted into indicator bacteria--where mutant genes can be measured (Winn et al. 1995, Winn et al. 2000, Winn 2001, Winn 2001a, Winn et al. 2001)	Industrial uses; Environmental uses	Research; method has been patented
Atlantic salmon	Increased growth rate and food conversion efficiency [Chinook salmon growth hormone gene	Aquaculture for human food	Method has been patented; initiated process of

	driven by antifreeze gene promoter] (Cook et al. 2000, Hew and Fletcher 1996)		seeking U.S. FDA approval for commercial use
Zebrafish	Flourescent red or green body color (Gong et al. 2003, Yorktown Technologies 2005)	Ornamental fish market	GloFish™ variety is for sale in USA
Red Sea Bream	Increased growth rates [ocean pout antifreeze protein gene promoter and Chinook salmon growth hormone gene] (Zhang et al. 1998)	Aquaculture for human food	Research
Rainbow Trout	Improved carbohydrate metabolism after [human glucose transporter type I and rat hexokinase type II gene, linked to viral (CMV) and piscine (sockeye salmon metallothionein-B and histone 3) promoters]. Could improve ability of fish to digest plant materials in formulated feeds. (Pitkanen et al. 1999)	Aquaculture for human food; Industrial uses	Research
Rainbow Trout	Increased growth rate and food conversion efficiency [via sockeye salmon growth hormone gene] (Devlin et al. 2001)	Model transgenic fish for public-domain research	Research
Carp and Medaka	Production of male-only offspring [altered gene that prevents the fish's aromatase enzyme from transforming reproductive hormone androgen into estrogen]. Lack of estrogen prevents development of female fish (Thresher and Bax 2003, Peacock 2004)	Biological control of aquatic nuisance species, such as common carp	Research; Medaka and other aquarium fish provide model for development of approach
Common Carp	Enhanced growth rate by [all-fish growth hormone gene and promoter sequences] (Zhu 2003)	Aquaculture for human food	Preparing to seek regulatory approval

Goldfish	Increased cold tolerance [ocean pout antifreeze protein gene] (Wang et al. 1995)	Aquaculture for human food	Research
Tilapia	Increased growth rate and food conversion efficiency [tilapia growth hormone gene construct] (Martinez et al. 2000, Pablo Estrada 2003, Lorenzo Herandez 2003)	Aquaculture for human food	Preparing to seek regulatory approval
Tilapia	Production of clotting factor [human gene for clotting factor VII] (Aquagene 2003, Hwang et al. 2004)	Pharmaceutical production	Research
Tilapia	Increased growth rate, food conversion efficiency, and utilization of protein [chinook salmon growth hormone gene linked to ocean pout antifreeze promoter] (Rahman et al. 2001)	Aquaculture for human food	Research
Mollusks			
Surfclam, Oysters	Potential improved disease resistance and growth acceleration in mollusks by harnessing altered genetic material from a virus to introduce foreign DNA [pantropic retroviral vector] (Lu et al. 1996, Burns and Chen 1999, Burns and Friedman 2002)	Aquaculture for human food	Research; method has been patented
Marine Plants			
Macroalgae	Enhanced production of carrageenan or agar after introduction of foreign DNA (Cheney and Duke 1995)	Ingredient in food, pharmaceutical, and cosmetic products	Research; method has been patented
Algae (*Spirulina*)	Potential improved nutritional and medicinal traits of commonly	Aquaculture for human food	Research

	consumed *Spirulina.* Transformation method confirmed by expression of marker gene (Zhang et al. 2001)		
Marine Microorganisms			
Diatoms	Reduced dependence on light for growth [human gene for biochemical involved in metabolism of sugar] (Zaxlavskaia et al. 2001)	Industrial uses	Research
Crustaceans			
Crayfish	Production of transgenic offspring (in crayfish and live-bearing fish) [replication-defective pantropic retroviral vector injected into gonads]. Transformation method confirmed by expression of marker gene (Sarmasik et al. 2001)	Aquaculture for human food	Research
Kuruma Prawns	Potential improvement of various traits. Insertion of marker genes to confirm gene transfer method (Preston et al. 2000)	Aquaculture for human food	Research

Biosafety science, policy, and regulation also need to address food and human health safety of genetically engineered fish that could intentionally or unintentionally enter the human food supply. Although beyond the scope of this paper's focus on environmental biosafety, food safety was the focus of a recent expert consultation convened by the United Nations Food and Agriculture Organization and the World Health Organization. The consultation's report reviewed the status of the science for assessing the safety of foods derived of genetically engineered animals, including fish, and issued a number of recommendations (FAO/WHO 2003). The report addressed environmental aspects of transgenic fish only in terms of the connection between environmental entry of transgenic fish and food safety.

Risk assessment and management

The science of biosafety for genetically engineered organisms develops methodologies and generates empirical data needed for risk assessment and management. Assessing and managing the environmental risks of aquatic GEOs requires integrating methods and knowledge from multiple fields such as genetics, physiology, evolutionary biology, population biology and ecology, community ecology, ecosystem ecology, and system safety science. Many mature industries use the elements of risk assessment and risk management processes, which are discussed below, to assess and verify the safety of their various technologies (Aldrich 1997, NRC 1996, McIntyre 2000, Amendola 2001, Kapuscinski et al. 2003). Risk assessment of transgenic organisms should follow a similar systematic process of distinct steps that build upon each other and lead to verifiable conclusions of safety or risk. Risk assessment of complex technologies often involves applying a mix of qualitative and quantitative methodologies (Apostolakis 2004, Burgman 2005), and this is also appropriate for assessing GEOs (NRC 2004).

Case-by-case approach

There is broad scientific and policy agreement that risk assessment and management of GEOs needs to be case-specific. A case-by-case approach should consider the characteristics of the non-engineered parental organism, inserted transgenes, altered traits of the GEO (including target and non-target traits), intended uses of the GEO, and accessible environments (i.e., environments that the GEO might intentionally or unintentionally enter through natural movements or anthropogenic means of transport) (ABRAC 1995, Scientists' Working Group on Biosafety 1998, NRC 2002, 2004). A key example of policy enshrining the case-by-case approach is the international Cartagena Protocol on Biosafety, in its "Annex III Risk Assessment" (Secretariat of the CBD 2000).

Systematic steps of risk assessment and management

Table 2 summarizes the systematic steps in risk assessment and management. Risk assessment involves hazard identification and risk analysis; risk analysis includes estimating exposure to the hazard, risk of harm given exposure to the hazard, and severity of harm. Risk assessment should also involve evaluating how well established is the knowledge used for each of these steps. This allows explicit consideration of the limits to quantifying risk, an issue discussed below in a section on policy implications. Deciding what constitutes an environmental harm and assessing the severity of potential harms may involve economic and social, as well as biological, considerations. The steps in risk management include risk reduction, risk monitoring and remedial action. Finally, risk communication should consider transparency and public participation in all steps of risk assessment and management, as discussed below in a section on policy implications.

Table 2. Systematic steps in risk assessment and management (Based on NRC 2004 and Kapuscinski 2002).

Step	Key Questions
Risk assessment	
Hazard identification	What event posing harmful consequences could occur?
Risk analysis	How likely is the hazard?
	What would be the harms from realization of the hazard, and how severe are they, taking into account social values?
	How likely is the harm, given hazard exposure?
	What is the risk assessment, as shown on a matrix of risk (likelihood of harm) plotted against severity of harm? (See Figure 1. Each cell of the matrix should be accompanied by a qualitative assessment of the response and a quantification of assurance needed to reduce harm if the cell's conditions were to occur.)
	How well established is the knowledge used to identify the hazard, estimate its risk, and predict harms?
Risk management	
Risk reduction planning and implementation	What can be done (including bioconfinement and other confinement) to reduce risk, either by reducing the likelihood or mitigating the potential harms?
	What steps might be taken to prepare for remediation?
Risk tracking (monitoring)	How effective are the implemented measures for risk reduction?
	Are they as good as, better than, or worse than planned?
	What follow-up, corrective action, or intervention will be pursued if findings are unacceptable?
	Did the intervention adequately resolve the concern?
Remedial action	

	What remedial action should be taken?
Risk Communication	
Transparency and public participation	How transparent should the entire process be? How much and what type of participation should there be in the steps above (and in risk characterization) by the public at large, by experts, and by interested and affected parties?

Risk assessors can summarize the risk assessment as a matrix of risk (likelihood of harm) plotted against severity of harm. Figure 1 depicts a simplified risk assessment matrix (Miller et al. 2004, NRC 2004). Depending on the quality of available information, the axes of a real risk matrix could consist of continuous values or more discrete categories. Social, economic, and ecological considerations influence the estimation of environmental harm and its severity. Great effort should be made to avoid approval of cases with high risk and most severe consequences (black area of Figure 1). Should the second level of priority be to address low probability hazards of high severity or high probability hazards with less severe consequences (the grey areas in Figure 1)? This question should be answered through deliberation among legitimate representatives of all potentially affected parties.

Status of science for risk assessment of genetically engineered fish

Of all the steps in risk assessment, the scientific community is best equipped to identify hazards posed by escapes of aquatic GEOs.

Hazard identification

Interdisciplinary teams of scientists have developed decision-support tools to identify potential hazards posed by the entry of transgenic fish into a natural ecosystem (ABRAC 1995, Scientists' Working Group on Biosafety 1998). Although some analysts consider the escaping fish to be the hazard (NRC 2002), these decision-support tools consider a hazard to be a phenomenon that can result from escapes of these fish. The entry of transgenic fish into a natural ecosystem could raise one of three major environmental hazards:

1. spread of transgenes to wild relatives of a native species;
2. spread of transgenes to feral relatives of an alien species already established in the ecosystem; or
3. heightened invasiveness by an alien species due to one or more traits altered by transgenesis.

For aquaculture applications, assessors can predict unintended entry of transgenic fish into natural waters by turning to empirical data sets on rates of escape of fish from different kinds of aquaculture operations, as well as practitioner knowledge; for instance, several recent reports review data on salmon escapes from net pens (NRC 2002, Pew Initiative on Food and Biotechnology 2003, NRC 2004a). The first and third hazards, listed above, would be the main concerns for intentional environmental introductions of transgenic fish, such as for biological control of invasive species. In such cases, assessors should also predict potential spread of the transgenic fish, beyond the targeted water body, to unintended water bodies. It would be important to consider all possible human and natural mediated means of spread (e.g., see table 1 in Scientists' Working Group on Biosafety 1998). Risk assessors should also look for GIS and other kinds of databases on native ranges of aquatic species, population status of native populations, and records of unintended introductions.

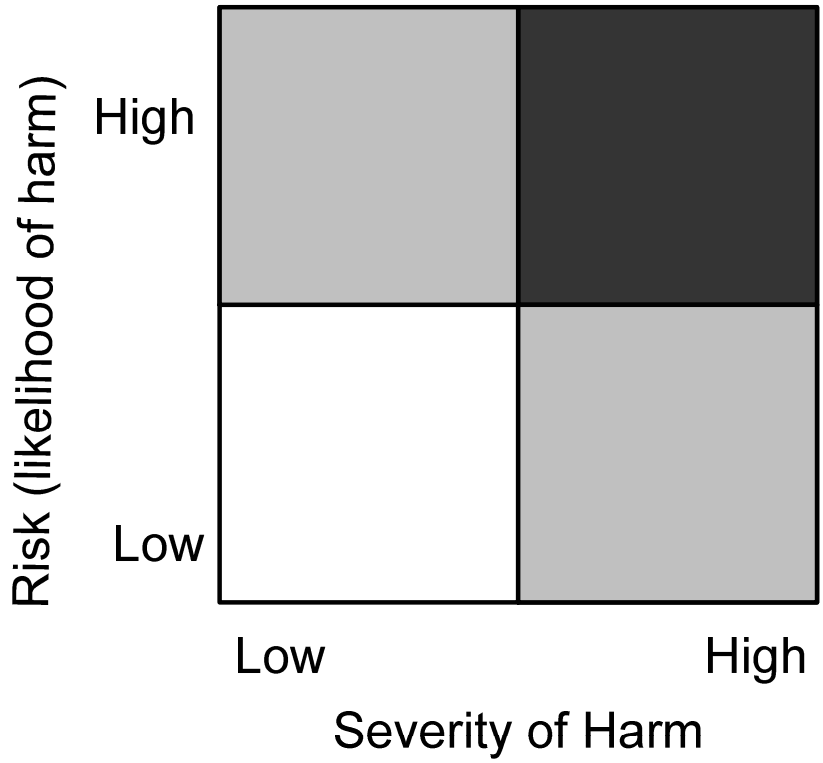

Figure 1. A simplified risk assessment matrix (based on Miller et al. 2004, NRC 2004).

Estimating exposure to the hazard with the net fitness methodology

To estimate hazard exposure, risk decision makers need a reliable, standardized, and sufficiently confirmed methodology consisting of tractable and repeatable tests that can

be conducted in confined settings and yield robust predictions. (Numerical models in the real world can only be *confirmed* to agree with relevant empirical data; verification – establishing that a model is 'true' – is impossible to achieve for open systems such as natural ecosystems; and validation – showing a model contains no flaws – is limited by existing knowledge about how the natural system actually works (Krebs 2003)) We do not yet have a methodology that meets all these criteria, but the net fitness methodology (Muir and Howard 2001, 2002) is one of the most promising candidates for getting to this point (FAO/WHO 2004, Hallerman 2002, Pew Initiative on Food and Biotechnology 2003, NRC 2002, 2004).

The net fitness methodology involves collection of fitness trait data on real transgenic individuals and their non-engineered counterparts, followed by input of these data into a mathematical model that predicts the fate of the transgene over multiple generations. Ideally the non-engineered counterparts should be from a truly wild population. The first step measures six fitness components in order to cover critical points in the entire life cycle of the organism and the second step quantifies the joint effect of all six fitness traits to predict transgene fate. To date, the methodology has been researched and discussed primarily with respect to the first two hazards listed above: potential spread of transgenes to (1) wild relatives, or (2) feral relatives. However, it could also be used to estimate exposure to the third hazard (heightened invasiveness by a transgenic alien species).

Studies with a growth-enhanced transgenic line of a model fish species, the Japanese medaka (*Oryzias latipes*) and model simulations in which transgenic fish escape into a population of wild relatives have shown that different combinations of values for six fitness traits could lead to three different predictions of transgene fate (Muir and Howard 1999, 2001, Pew Initiative on Food and Biotechnology 2003):

1. *Purge Scenario* - purging of the transgene at some time after initial escape of transgenic fish;
2. *Spread Scenario* - spread of the transgene through a wild population of relatives with no impact on the introgressed population's size; or
3. *Trojan Gene Scenario* –initial transgene spread that then triggers a decline in the introgressed population's size; occurs when the transgene has an antagonistic effect on different fitness traits.

Comparable scenarios for transgenic alien species would be (Pew Initiative on Food and Biotechnology 2003):

1. *Disappearance Scenario* – transgenic fish disappears from the environment when their net fitness is much lower than that of the parental species;
2. *Establishment Scenario* – transgenic fish establish a self-regenerating population when their net fitness is greater or equal than that of the parental species.

Purging/disappearance: Gong et al. (2003) modified the methodology for fitness trait measurements to generate data suggesting transgenic zebra fish (*Danio rerio*) with strong expression of a fluorescent protein gene in skeletal muscle would not pose a higher probability of invasiveness into non-native habitats than would conventional zebra fish. These findings were invoked in the recent commercial release of these transgenic zebra fish into the ornamental fish market in the United States (Gong 2003, Yorktown Technologies 2005).

Transgene spread: Muir and Howard (2001) suggested that age at sexual maturity has the greatest influence on the likelihood of transgene spread through a population of wild relatives, followed by juvenile viability, mating advantage, female fecundity, and male fertility. Some data consistent with predicted transgene spread were collected from studies of fast-growing transgenic lines of species relevant for food-production aquaculture. Coho salmon bearing a type-1 sockeye salmon growth-hormone gene driven by the sockeye salmon metallothionein promoter (*pOnMTGH1*) were, on average, eleven times heavier than conventional controls and had an earlier age at sexual maturity (Devlin et al. 1994), which is the most important fitness trait influencing transgene spread (Muir and Howard 2001). Larger size and younger sexual maturity was also found in one line of transgenic medaka bearing the *psGH-hGH* construct, consisting of a salmon growth hormone promoter linked to a human growth hormone gene (Muir and Howard 2001). Devlin et al. (2001) found that transgenic rainbow trout (construct *pONMTGH1*) started from a wild population had lower viability and 37-83 times larger size at sexual maturity than wild fish. Larger size at sexual maturity could give these trout a large mating advantage (Fleming 1996, 1998; de Gaudemar 1998), which is the third most important fitness trait affecting transgene spread. Transgenic tilapia bearing a Chinook salmon growth hormone gene driven by ocean pout antifreeze protein gene promoter (*OPAFPcsGH*) were three times larger than controls both as juveniles and at sexual maturity (Rahman and Maclean 1999). Predicting whether or not these transgenic fish lines fit the spread scenario requires obtaining complete net fitness measurements. So long as they exhibit earlier age at sexual maturity or larger size at maturity, these lines would have to exhibit severe reductions in viability to fit the safer, purging scenario.

Trojan gene effect: The growth-enhanced lines of transgenic salmon, trout, and tilapia discussed in the above paragraph could perhaps fit the Trojan gene scenario if their viability is moderately reduced (Muir and Howard 1999, 2002). Howard et al. (2004) documented that one of their growth-enhanced transgenic medaka lines exhibits reduced juvenile viability and a male mating advantage, with a mathematical prediction of transgene spread leading to eventual extinction.

The net fitness methodology needs to be confirmed by producing evidence that its predictions agree with empirical data for a test case. Towards this end, empirical tests are ongoing in the author's laboratory. The approach is to compare mathematical model predictions to the fate of transgenes after releasing transgenic medaka (*pMT-sGH*, provided by Bill Muir) into contained mesocosms where they have the opportunity to

interbreed with non-transgenic medaka in a multi-age class, naturally reproducing population. Substantial variation in transgene fate has been observed several generations post-release, both within and between two transgenic lines, in one completed and one ongoing release trial (data unpublished).

Several weaknesses of the net fitness methodology, in its current form, have been identified (Hallerman 2002). For instance, the present approach to measuring the six fitness traits ignores ecological factors that could affect the fate of the transgene. It is not feasible to make the net fitness methodology perfectly mimic all ecological factors in nature, nor is this necessary to make it a powerful tool for risk assessment. Indeed ecological modelers have learned that excessively complex models can become less rather than more reliable in their predictive power (Krebs 2003, Ginzburg and Jensen 2004). But it is important to strategically identify the most important factors that need to be added to greatly improve the reliability of its predictions of transgene fate. One priority should be to determine whether or not the six fitness traits in a given transgenic line are affected by genotype-environment interactions. One would want to know if transgenic fish exhibit lower net fitness than wild-types under one value of a limiting environmental factor but a higher net fitness under another value, because this could switch predictions of transgene fate from purging to spread. If appropriate tests show strong genotype by environment interactions, it would be relatively easy to add an appropriate algorithm to the mathematical model. The mathematical model also lacks a function for stochastic processes that could affect transgene fate and it assumes no adaptive evolution in fitness traits. These and other identified weaknesses of the current methodology could be addressed through appropriate data collection and revisions of the mathematical model. A number of research teams are tackling these issues.

Assessing environmental harm and its severity

The scientific ability to assess the consequences of ecological spread of aquatic GEOs is the weakest of all the steps in risk assessment. This phase of risk assessment involves identifying possible environmental harm, estimating the risk (likelihood of harm given occurrence of a specific hazard), and assessing the severity of the harm, taking into account social values for the affected part of the environment. Simply put, this phase addresses the pivotal 'so what?' questions: if transgenic fish escape and spread their genes through a wild population, will this really harm the environment and, if so, what will be the harm? More than any other step in risk assessment, environmental harm assessment requires scientists to integrate analytical methods and information across numerous scientific fields, such as population and conservation genetics, evolutionary biology, population biology (especially conservation biology methods such as population viability analysis), population ecology, community ecology, and ecosystem ecology.

Interdisciplinary scientific teams have identified different possible environmental harms, depending on the characteristics of the transgenic aquatic organism and the affected aquatic ecosystem (ABRAC 1995, Scientists' Working Group on Biosafety 1998, NRC

2002, 2004). These potential harms can be grouped under one of three broad categories. Listed below in increasing order of difficulty to make scientifically reliable assessments, they include possible harm to:

1. gene pools in the affected species' center of origin;
2. species of special concern, such as endangered, economically or culturally important species; and
3. ecological resilience of fish communities—their ability to recover from external disturbances, such as floods, contaminants, or climate change.

Some decision-support tools can help identify the case-specific issues to consider in assessing these possible environmental harms. For example, portions of the *Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organism*s (Scientists' Working Group on Biosafety 1998) can assist assessment for all types of aquatic GEOs. There is a desperate need, however, to establish standardized, scientifically vetted methodologies for assessing these broad categories of environmental harms.

Environmental harm assessments should also consider potential genotype-environment interactions, such as those that have been demonstrated in laboratory research on food competition between growth-hormone transgenic and unmodified coho salmon (Devlin et al. 2004). For example, mixed populations of transgenic and non-transgenic salmon experienced population crashes or complete extinction under environmental conditions of low food-availability, but maintained a stable population size under conditions of high food-availability.

The hazard scenario determines the categories of ecological harm that should be assessed (Table 3). The purging and disappearance scenarios are the environmentally safest options but may not always be impact free. Purging of maladaptive transgenic fish by natural selection is not instantaneous but would occur over a number of generations depending on the degree of natural selection against the transgenic phenotypes. When potentially affected wild populations are already in decline, it would be necessary to assess the potential for harm to species of special concern. When assessing possible harm from spread of a transgene, it is relatively easy to predict effects on gene pools in centers of origin, somewhat harder to predict effects on species of special concern, and extremely difficult to predict effects on resilience of fish communities (Pew Initiative on Food and Biotechnology 2003). Assessing potential harm from the Trojan gene effect is more straightforward because its predicted population decline constitutes an environmental harm. Loss of a wild fish population would clearly lead to loss of unique genes. If transgenes conferring the Trojan gene effect spread through a threatened or endangered population, this would increase its chance of extinction. And loss of an entire population might reduce fish community resilience, for instance through simplification of the food web, unless the community contains other species that serve the same ecological

function. Current scientific understanding of ecological resilience was reviewed by the International Council for Science (2002).

Table 3. Environmental hazard scenarios determine the categories of ecological harms that should be assessed. Scientific difficulty of assessing harms increases going from the left to right columns.

Hazard scenario	**Assess potential ecological harms**				
	Predicted fate of transgenic individuals	Ecologically safe?	Alter genetic diversity?	Harm species of special concern?	Reduce aquatic biotic community resilience?
Gene flow to wild relatives					
	Purging	Assess		Assess	
	Spread		Assess	Assess	Assess
	Trojan gene		Assess	Assess	Assess
Alien species invasion					
	Disappearance	Assess		Assess	
	Establishment			Assess	Assess

Very little thought has been given to the potential effect of genetically engineered organisms on ecological resilience but this could become the 'million dollar question' if GEOs come into widespread use. Other human causes of declines in ecological resilience have been characterized by long lag times before the harm was documented (International Council for Science 2002). Loss of productivity and ecological resilience has happened after long-term enhancement of single species in fisheries (Gunderson et al. 1995). Potential harm to ecological resilience due to widespread or large-scale uses of transgenic organisms would be difficult to predict in small scale, regional field trials or laboratory experiments. Post-commercialization testing and monitoring research, therefore, would be needed to learn whether or not this is a serious problem when producing transgenic organisms in different aquaculture systems.

Status of science for risk management of genetically engineered fish

Risk management in many technology industries entails risk reduction planning and implementation, post-release monitoring and remedial action as outlined in Table 2 (Kapuscinski 2002, Kapuscinski et al. 2003, NRC 2004). To date, discussions about risk management of transgenic fish have focused on risk reduction and largely ignored risk monitoring and remedial action. It is also unclear if regulatory agencies in different countries intend to stipulate risk monitoring and remedial action plans as part of any approvals they might give for large-scale production of transgenic fish.

Discussions and proposals for risk reduction have focused on confinement methods that would reduce environmental entry of transgenic fish and spread of their transgenes. A recent report on biological confinement of genetically engineered organisms, commissioned by U.S. Department of Agriculture, had a number of findings and recommendations applicable to risk management of transgenic fish (NRC 2004). Findings particularly relevant to transgenic fish include:

• Confinement of transgenic organisms can be accomplished physically (e.g., by screens and other mechanical barriers to escape from fish tanks and ponds), physico-chemically (e.g., by lethal water temperatures or chemicals applied to water existing fish tanks), or biologically (e.g., by rendering the organism incapable of reproducing or of surviving outside of the aquaculture system).

• The best developed and scientifically documented method for biological confinement of transgenic fish involves disruption of sexual reproduction by triploidy induction.

• Weaknesses of triploid sterilization include incomplete success of producing triploids and that the degree of functional sterility in triploids varies depending on the species and sex.

• If large numbers of triploid transgenic individuals were to enter the environment on a recurring basis, it would be necessary to assess ecological harm because triploids of some species have enough sex hormones to cause them to engage in normal courtship and spawning behavior, which could lead to losses of entire broods and lowering reproductive success of wild fish. It would also be necessary to assess possible heightened predation or competition by sterile transgenic adults if they survive and grow for an indeterminate period beyond the normal life span.

• Combining triploidy with all-female lines in some fish species such as salmon can increase the degree of functional sterility.

• Less than total triploid induction can be mitigated by mass screening methods to identify and remove non-triploids before they are transferred from more secure hatcheries to less secure grow-out facilities, such as outdoor ponds or open water cages. Detection limits and operator error are facts of life with these screening methods.

• Deciding whether to screen every individual destined for grow-out or only a sample of each production lot should consider the risk and severity of harm (Figure 1) and the extent to which adequate additional confinement measures are in place.

• Within the native range of Atlantic salmon, the primary and unresolved ecological concern is whether the movement of transgenes into endangered wild populations would

further depress their fitness. Individual screening followed by culling of non-triploids would be the prudent choice for farming all-female, triploid Atlantic salmon in open-water cages in areas—such as the Maine coast—where wild populations are already depleted severely. In salmon farming areas outside the natural range of Atlantic salmon, the main question would be whether the net fitness of transgenic Atlantic salmon is higher or lower than currently farmed strains and thus whether the transgenic fish would be more or less of a threat to become an invasive species.

• A conservative estimate indicates that the cost of screening individual salmon by flow cytometry would add U.S. $0.02 to $0.04 per 1 kg of fish to the cost of harvested adult Atlantic or chinook salmon (Kapuscinski 2002). This estimate is conservative because it is based on small-scale tests, does not account for feasible economies of scale, and does not include cost-savings that could be achieved by computer automation of screening steps.

• There are a few well documented cases of sterile inter-specific hybrids. One study, involving two consecutive but different crosses of hybridization between carp species, reported highly effective sterility of progeny of the second cross (Liu et al. 2001). It has been proposed as an ideal way to biologically confine transgenic carp (Zhu 2003).

• Biological confinement of transgenic fish may be possible by gene blocking, gene knockout, and externally administered gene-specific compounds but these methods are at very early stages of research.

• It is unlikely that 100% confinement will be achieved by a single method.

• Redundancy in confinement methodology will increase the chances of attaining the desired confinement level (eg, combining triploidy with all female lines and farming transgenic fish in units with high levels of physical confinement).

The report's recommendations are as follows (NRC 2004):

1. Evaluation of the need for bioconfinement should be considered for each GEO separately.
2. The need for bioconfinement should be considered early in the development of a GEO or its products.
3. Confinement techniques should be tested experimentally, separately and in combination, in a variety of appropriate environments, and in representative genotypes under development before they are put into application.
4. To evaluate changes in reproductive biology, the novel genotype should be compared with that of its progenitor before field release.
5. Bioconfinement techniques should be assessed with reference to the temporal and spatial scales of field release.

6. An adequate level of bioconfinement should be defined early in the development of a GEO, after considering worst-case scenarios.
7. An integrated confinement system approach should be used for GEOs that warrant confinement. The stringency of the integrated confinement system should reflect the predicted risk and severity of harm of GEO escape. Elements of an integrated confinement system include:
   - Commitment to confinement by top management;
   - Establishment of a written plan for redundant confinement measures to be implemented, including documentation, monitoring, and remediation (in case of failed confinement);
   - Training of employees;
   - Dedication of permanent staff to maintain continuity;
   - Use of standard operating procedures for implementing redundant confinement measures;
   - Use of good management practices for applying confinement measures to pharmaceutical-producing GEOs or the equivalent;
   - Periodic audits by an independent entity to ensure that all elements are in place and working well;
   - Periodic internal review and adjustment to permit adaptive management of the system in light of lessons learned;
   - Reporting to an appropriate regulatory body.
8. Easily identifiable markers, sampling strategies, and methods should be developed to facilitate environmental monitoring of GEOs.
9. Transparency and public participation should be important components in the development and implementation of bioconfinement techniques and approaches.
10. The possibility of human error should be taken into account as a factor when determining bioconfinement methods and evaluating their efficacy.
11. Regulators should consider the potential effects that a failure of confinement could have on other nations, as well has how foreign confinement failures could affect the United States. (This point could apply to any nation.)
12. International cooperation should be pursued to adequately manage confinement of GEOs.

Implications for policy

The above discussion showed that scientific methods and data are better developed for some steps of environmental risk assessment and management of aquatic GEOs than for others. It also identified some critically important gaps in the scientific tools and information needed to reach verifiable conclusions at key steps in risk assessment and management. These findings raise a number of implications for policy and regulations that aim to govern safe uses of aquatic GEOs.

Limits to quantification in risk assessment

Risk is often described as a formula: the probability of hazard exposure multiplied by the conditional probability of harm, given that exposure has occurred. Although it can be very useful to attempt to quantify risk via such a formula, risk is not easily estimated. To quote a recent NRC (2004) report, "Uncertainties can arise from random events (including human error) in the physical world, lack of knowledge about the physical world, or lack of knowledge about the applicability of risk-generating processes (NRC 1996). Ecological harm is difficult to quantify, including damage to an ecosystem or the extinction of a species, for example. Moreover, evaluating harm requires consideration of social values that will define the significance of predicted consequences. Clearly, exact quantification is impossible."

Efforts to assess the environmental risks of a given transgenic fish do not need to be paralyzed by these limitations in quantifying risk. They would benefit, however, from explicit recognition and thorough consideration of these limitations during the process of risk assessment and management. This should include incorporation of techniques to address different sources of uncertainty (Burgman 2005). For instance, building stochasticity into predictive models, such as the net fitness mathematical model, would provide one way to resolve some of the uncertainty arising from random events. The application of Bayesian analytical methods would go a long way towards addressing the degree to which lack of knowledge about natural processes involving fish in aquatic ecosystems would alter predictions of safety or risk (Malakoff 1999).

Cumulative risk

Cumulative risk assessment is a major challenge for risk decision making about GEOs. There has been little consideration of cumulative risk assessment for aquatic GEOs primarily because they are not yet in wide use. The intent of most commercial applications is to achieve widespread adoption of the aquatic GEO, suggesting that risk decision makers will likely eventually need to assess cumulative risks. Large-scale production of one or more lines of transgenic fish, for instance, could lead to multiple environmental entries of the transgenic fish across time and space. Methods for cumulative risk assessment have not been well developed for assessing the effects of various technologies in aquatic environments. Nevertheless, the issue is gaining prominence in many aquatic environment policy arenas. For instance, cumulative risk assessment is one part of an aquaculture environmental assessment tool developed for the Great Lakes Fishery Commission (Brister and Kapuscinski 2001).

Choice of appropriate alternative technology for comparing risks

Selecting the most appropriate alternative technology for comparison of risks posed by GEOs is another challenge facing risk decision makers (NRC 2004). Although this issue has received considerable attention for transgenic crops (NRC 2002a), it has not been

adequately addressed for transgenic fish and other aquatic GEOs. There is a need to develop appropriate comparisons of transgenic fish to other options for meeting such objectives as increasing fish farm yields, reducing disease outbreaks on fish farms, or effectively controlling invasive fish species in natural water bodies. It will be important to avoid too narrow a choice of appropriate alternatives for comparison. Major aquaculture industries are undergoing some changes which will likely change the production strategies and practices of many firms. One sign of change is the involvement of diverse parties from the aquaculture sector in the development and promotion of organic aquaculture as a way to increase profits through environmentally friendly production practices (Brister 2004). The USDA National Organic Standards Board has started the process to draft organic standards for aquatic animals (Agricultural Marketing Service 2005). Similarly, nascent efforts to develop transgenic fish for biological control of invasive aquatic species are arising at a time when experts and savvy public stakeholders understand the pitfalls of relying on one 'silver bullet' solution. Instead, they are stressing integrated pest management approaches that might place heavy emphasis on habitat rehabilitation, species-specific chemical controls, and even the use of species-specific fish pheromones.

Interdisciplinary scientific analysis

The process of assessing and managing environmental risks of aquatic GEOs will remain challenging for a long time to come. The scientific reliability of risk decisions depends heavily on the degree to which they involve a "process of appropriate [scientific] scope, covering ecology and other relevant life sciences (getting the right science) and of reliable content (getting the science right)" (Kapuscinski 2002). This will require a serious commitment to interdisciplinary risk analysis, going beyond simple desk reviews of literature. It would be much more effective to form interactive teams of risk analysts trained in different fields. In most cases, such an interdisciplinary approach should include involvement of experts in genetics (from molecular to quantitative and population genetics), animal physiology including behavior, environmental physiology, evolutionary biology, population biology and ecology, community ecology, ecosystem ecology, and system safety science and management. Also needed are policies that allow and encourage independent scientists from different fields to review the quality of the analytical approach and data being considered long before regulators make a final risk decision.

The biotechnology industry has largely ignored the field of system safety science and management. In doing so, this relatively new industry is missing the chance to benefit from the pro-active safety wisdom gained by mature industries whose technologies also offer societal benefits while sometimes posing risks of public harm (Kapuscinski et al. 2003, Kapuscinski 2003a). System safety science and management is a well established set of principles and practices that stress accident prevention and learning from mistakes via scientific and participatory processes, starting at the earliest stage of design through

research, development, and final use of a particular technology (McIntyre 2000, Kirwan 2001, Baram 2002).

Need for clear standards of environmental safety and harm

In the United States, policies and laws that might be used to govern the environmental safety of transgenic fish lack explicit standards for environmental safety (Pew Initiative on Food and Biotechnology 2003). This is like trying to steer a ship without knowing the intended destination. Experience from other industries strongly suggests that establishing explicit safety standards through a participatory, open, and interdisciplinary process and then focusing biotechnology product development and use, as well as regulatory policy, on meeting these standards would have multiple benefits. It would make government oversight more effective and less burdensome, increase chances of earning consumer and public confidence, reduce litigation and insurance costs, and improve business viability (Kapuscinski et al. 2003). The first step would be to bring together parties from the business, public interest, academic, and government sectors to negotiate clear environmental safety objectives that forthcoming proposals for commercial uses of aquatic GEOs would have to meet. Recent efforts in this direction include a cross-sectoral team that is developing a 'safety first' approach in the United States (Kapuscinski et al. 2003), and an international workshop convened in Thailand in 2003 to explore the issues involved in designing safety standards for transgenic fish.

Representative deliberation: essential for acceptance of risk decisions

The development of aquatic GEOs for various commercial uses is occurring at a time when the social contract between science and society is undergoing a major transformation. Instead of expecting science to produce 'reliable' knowledge and then communicate its findings to society, this new social contract expects science and its technological applications to be 'scientifically reliable' and 'socially robust', as well as open and transparent, in order to gain broad acceptance (Gibbons 1999). Decisions and actions regarding the biosafety of specific GEOs therefore require greater integration of science with participatory processes and policy than in the past. In recent successful cases of taking a 'safety first' approach to uses of technology (Kapuscinski et al. 2003), "an analytic-deliberative process has evolved whereby potentially affected parties in the private and public sectors collectively identify key safety issues to be addressed, which in turn has produced knowledge and agreements about safety that met and moved beyond scientific 'reliability' to 'socially robust' and publicly credible arrangements (Gibbons 1999)". Other examinations of the successes and failures at winning durable public trust of risk decisions have reached similar conclusions (NRC 1996, Sagar et al. 2000, Marris et al. 2001, Nowotny et al. 2001).

The best way to reach broad and long-term social acceptance of risk decisions and potential approvals of aquatic GEOs will be to involve potentially affected parties in representative and transparent deliberation of scientific and technical information at early

to late stages of the risk assessment and management process. The deliberative process is particularly important for helping to identify the environmental harms that matter most to potentially affected parties and that should be addressed in the risk assessment. Adequate representation of potentially affected parties in deliberation processes is also essential for putting on the table the relevant social, economic, and other non-biological factors to consider in assessing potential harms and their severity. These ideas could be implemented through policy innovations that fit a particular nation's societal context.

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Participant Discussions

Fletcher: Before we started aquaculture, if we had applied this risk assessment methodology, would there be an aquaculture industry at all?

Kapuscinski: Yes, an industry would exist. I think that if the public had been involved initially, the public would have been more on board, and aquaculture would likely have evolved differently in a more ecologically sound and publicly accepted way. There is always a better chance of getting public acceptance by using a more systematic approach. A few years ago some sociologists were contracted to do a study of public views of biotechnology in Europe (Marris et al. 2001, cited in my paper for this meeting). It's really interesting to read the results of that report. They did interviews, focus groups and surveys in five European countries. The public is not as naïve as we think about risks. The people interviewed were also pretty savvy about kinds of uncertainty. They want the risk assessment process to be more transparent and to see that the regulatory bodies are doing the best they can with the information we have. But they also want risk decision makers to engage the uncertainties. Even though they may not agree with each individual decision, they would be comfortable with an improved process that seriously considers uncertainties and they would tend to agree with the bulk of decisions from such a process.

SOCIAL/ENVIRONMENTAL ISSUES REGARDING GENETICALLY ENGINEERED FISH USE: POLICY CONSIDERATIONS

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Abstract

Regulatory policies to address the potential risks associated with genetically engineered aquatic animals must operate in a socioeconomic as well as a scientific/technical context. Based upon field interviews in British Columbia about possible use of transgenic fish in salmon farming, I suggest that social resistance comes not only from activists and scientists concerned about potential effects on human health or ecological functions but also from aquaculture producers concerned about consumer reaction and structural changes to their industry. This resistance has created a socioeconomic space in which to adopt policies that accomplish their purposes while achieving public support for the regulatory outcomes. Public perception of environmental risk comes, in part, from scientific uncertainty about the impacts on ecological functions if transgenic fish are introduced into ecosystems. The current US federal regulatory approach inadequately addresses scientific uncertainties and perceptions of ecological risk because it neither directly engages the regulatory agencies that have the greatest expertise in fisheries and ecology nor does it provide a transparent process through which independent scientists and the public can participate meaningfully. Canada has the opportunity to avoid those problems and to establish a transparent federal regulatory approach based upon open discussion of the scientific and socioeconomic issues.

Résumé

Les politiques de réglementation élaborées afin de gérer les risques que pourraient présenter des animaux aquatiques génétiquement modifiés doivent être appliquées dans un contexte à la fois socio-économique, scientifique et technique. Sur la base d'entrevues effectuées sur le terrain en Colombie-Britannique et qui portaient sur l'utilisation possible de poissons transgéniques en salmoniculture, je suggère que la résistance sociale n'est pas seulement le produit d'activistes et de chercheurs qui s'inquiètent à propos des effets possibles sur la santé humaine ou sur les fonctions écologiques, mais également d'aquaculteurs préoccupés par la réaction des consommateurs et des changements structurels dont devrait faire l'objet l'industrie. Cette résistance a créé un espace socio-économique dans lequel il est possible d'adopter des politiques efficaces tout en assurant l'appui du public à l'égard des décisions en matière de réglementation. La perception du public à l'égard des risques pour l'environnement est due en partie à l'incertitude scientifique en ce qui concerne les répercussions de l'introduction de

poissons transgéniques dans les écosystèmes sur les fonctions écologiques. L'approche de réglementation actuelle du gouvernement fédéral des États-Unis ne traite pas adéquatement des incertitudes scientifiques et des points de vue relatifs aux risques pour l'environnement parce qu'elle ne fait pas participer directement les organismes de réglementation qui ont le plus d'expérience dans le domaine des pêches et de l'écologie et qu'elle ne fournit pas un processus transparent grâce auquel le public et des chercheurs indépendants pourraient participer de façon concrète. Le Canada a la possibilité d'éviter ces problèmes et d'établir une approche de réglementation transparente fondée sur une discussion ouverte sur les questions scientifiques et socio-économiques.

Introduction

In North America, the policy discussion about the use of genetically engineered (GE) aquatic animals has tended to focus on relatively narrow technical issues of human health and environmental effects. However, the socioeconomic concerns are much broader and so is social resistance to certain uses of biotechnology. For example, in the conflicts over genetically engineered foods, issues of control over both food supply and producers are important subtexts. In the words of a British Columbia activist:

> I'm unclear as to what the risks [of transgenic salmon] are, ... but it offends me that this source of protein that we've got—the last source of protein from the wild—is being taken over by big business. There are huge social implications of this privatization of what was a wild resource. Somebody in a little coastal community is now the serf in the corporate kingdom (Interview 1999.40).

Diverse sources of resistance have been documented in the social science literature (see, e.g., Schurman and Munro, 2003). Many of these concerns are beyond the scope of this meeting, but it is important to recognize the breadth of socioeconomic issues in order to understand why controversy may occur even if technical questions are resolved.

In the first part of this presentation, I focus primarily on social resistance to GE food fish from the perspective of the salmon aquaculture industry in Canada. Salmon farmers' resistance reflects not only industry sensitivity to consumer reaction but also other public perceptions of the risks associated with non-medical applications of GE animals.[i] I argue that this resistance has created the socioeconomic "space" in which to adopt effective regulatory policies. Second, I examine the current regulatory framework in the United States and suggest that those policies have limited effectiveness because they lack transparent processes and substantive standards that are applied "upstream"— well before consideration of deployment proposals. By "effective policies," I mean policies that accomplish their regulatory purposes and that achieve broad public acceptance of regulatory outcomes. The term "transparent processes" refers to open and meaningful involvement of the public, including scientists, in regulatory decisions about GE fish.

Aquaculture industry resistance

The industry's resistance arises primarily from three clusters of concerns: consumer reaction, environmental risk, and effects on existing industry structure. For purposes of my discussion, I assume that the growth rate increase attributed to GE Atlantic salmon is an attractive production advantage. As an aquaculture trade association official put it, "There's no doubt that transgenic fish grow faster," the question is whether these salmon will be acceptable to regulatory agencies, salmon farmers, and consumers (Interview 1999.28).

Aquaculture trade organizations have overwhelmingly taken the position that transgenic fish are a bad idea for the industry at this time. The British Columbia Salmon Farmers Association (2000) has announced its opposition to transgenic fish "unless science can prove that they present no danger to . . . wild stocks or the marine environment." Similarly, the International Salmon Farmers' Association policy states, "In accordance with sound environmental practise, the [International Salmon Farmers' Association] firmly rejects transgenic salmon production" (International Salmon Farmers' Association 2000).

The battle for public perception

Aquaculture producers fear that as a result of controversy about GE food fish, the public would reject not only the transgenic product but also farmed salmon generally, whether selectively bred or transgenic. The Province of British Columbia's Salmon Aquaculture Review expressly recognized this concern:

> Currently, there are no transgenic salmon being farmed commercially in BC, nor has there been any interest expressed for doing so. This is primarily related to the negative public perception of transgenics, and the potential for this to affect all farmed salmon sales (Environmental Assessment Office 1997, v. 1, p. 81).

The aquaculture industry has achieved profitability by using technologies to control natural production processes and by securing substantial consumer acceptance of those technologies. Without a compelling reason, salmon farmers are reluctant to adopt an innovation that is likely to raise questions in the minds of consumers.

The industry's concern about consumer reaction is underscored by attacks on farmed fish as either unhealthy (e.g., by calling attention to use of pharmaceuticals in the production process) or environmentally harmful (e.g., by calling attention to waste discharges and other grow-out site practices). A recent article in *Science* fueled the arguments of some activists by identifying relatively high concentrations of organic pollutants in farmed fish sampled from different producing regions around the world (Hites et al. 2004). A British

Columbia aquaculture scientist summarized the industry's concern about consumer reaction to transgenic fish:

> [T]his polarization, where one's good and one's bad, [genetically engineered salmon] would just enshrine that, I'm afraid. ... So, it could be a good technology; and it could have a real price tag on it in terms of public support for the industry (Interview 1999.26).

Referring to the controversy over a different technology—bovine somatotropin—he extended consumer concerns, by analogy, to salmon:

> [J]ust looking at it pragmatically, [bovine growth hormone] hasn't been a very smooth technology to introduce to the public. There's a lot of reaction. And so, I don't have a good feeling for what would happen with transgenic fish on farms, in terms of public perception. Certainly, the critics of salmon farming would leap on that, and it would become a huge issue ... (Interview 1999.26).

Nevertheless, the aquaculture industry depends upon technological innovation to control natural processes, to reduce costs, and to gain other competitive advantages. As long as the resulting commodities are acceptable in the market place, production technologies that lead to cost savings or quality improvements may strengthen the adopter's short-term competitive position in relation to other salmon farmers who have not implemented those technologies. However, it is this short-term, first-mover advantage that also increases the concerns of aquaculture producers about possible impacts on the structure of the industry.

Perceived structural risks to the existing aquaculture industry

Salmon farmers fear destabilizing loss of control at several levels. Competitive advantages in commercial salmon farming are now based upon conventional technologies such as selective breeding programs, high-performance feeds, computer-controlled automation, and other on-site mechanization. Salmon aquaculture companies have invested in the development of artificially selected, proprietary brood stocks to increase growth rates and enhance other characteristics important for production or marketing. Vertically integrated salmon farming companies and specialty suppliers of smolts have invested heavily in broodstocks that achieve substantial increases in growth rates (as well as other performance characteristics) compared to wild fish of the same species (Interview 1998.05; Isaksson 1991).

Because patented transgenic fish would be available only to those producers licensed by the holder of the patent, some producers might gain access to the technology while others do not. In addition, aquaculture companies might lose the competitive advantages they have obtained by investing in the development of faster growing fish through artificial selection programs. In production areas like North America's East Coast, which compete

in markets that are aggressively served by Chile—a production region with lower costs of production—current producers would be under intense price pressure if they do not (or cannot) adopt the technology while their competitors successfully deploy transgenic fish.

Writing about adoption of new technologies in agriculture, sociologist Jack Kloppenburg (1988) described a "technology treadmill" that reduces unit costs of production and enforces adoption of process changes in a tightening spiral:

> Early adopters of new technologies enjoy windfall innovators' rents, but these disappear as adoption spreads and the cost curves for all operations converge. Because the adoption of new technologies results in increased production, there is a tendency for prices to fall. This merely sets the stage for another round of innovation (p. 35).

This treadmill not only speeds the producer toward adoption of technology, it also impels the system toward "cannibalistic centralization," as marginal producers—those unable to adopt new technologies—are absorbed by other operators or disappear entirely. This leap-frogging of first-mover advantage followed by industry restructuring is a major concern for the aquaculture industry and a source of its resistance to GE Atlantic salmon.

The benefits of output-increasing genetically engineered organisms, whether fish or crops, will go primarily to the firms that own the patented organisms and to the producers who are among the first to adopt the technologies. If genetically engineered fishes increase production, they (like other yield-enhancing agricultural technologies such as Bt corn) will generate more output in the aggregate. In the face of inelastic demand (e.g., for staple agricultural commodities), this growth in output disproportionately reduces prices and profitability for all producers, except to the extent that public subsidies protect producers from these effects (Harl 2003).

Salmon growers describe a powerful momentum pushing producers from technology to technology, raising anxiety about being left behind in the competition for lower production costs and being overtaken by falling prices. In the words of a British Columbia aquaculture trade association representative:

> Nobody can resist lower cost production. Certainly if somebody comes up with a way of doing it and you sell it for a dollar a pound less than conventionally reared salmon, either the rest of the places in the world will have to meet that or they'll go out of business. (Interview 1999.25).

Salmon farmers expect, and fear, that once a salmon aquaculture company successfully initiates the use of transgenic fish somewhere in the world, others will adopt the technology in order not to be left behind. The combination of improvements in production efficiency, opportunities for competitive advantage, and expectations of spiraling adoption pressures would seem to support the conventional wisdom that a

transgenic treadmill and consequent structural change may be compulsory for the industry.

However, this assumption is undercut by the escalating social struggle that has accompanied the development of the new transgenic commodities. Social resistance has focused initially on consumer concerns about the safety of genetically modified foods. Despite the initial focus on consumer acceptance of GE food animals and the prominent discussion of that issue in both the trade press and the news media, salmon farmers suggest that food safety will ultimately not be the most serious problem. Instead, salmon farmers indicate that ecological risk will likely prove to be the largest challenge (Interview 1998.14).

Scientific uncertainty and public perceptions about environmental risk

The public's perception of ecological risk responds, in part, to uncertainty among scientists about what will happen if transgenic organisms escape or are released intentionally into natural ecosystems. Also contributing to the controversy is the documented, persistent problem of farm fish escaping from floating net pens into surrounding waters and then traveling long distances from the point of escape. For salmon farmers, escaped fish are the most prominent and difficult issue in a suite of claims by the industry's opponents about environmental harms from salmon aquaculture.[ii] If genetically engineered salmon are deployed in marine net pens, escapes of these fish will be the most important factor in their potential to cause ecological harm.

Salmon farmers identify two dangers to the industry from environmental questions about transgenic fish. First, they fear that public perception of environmental risk could fuel additional opposition to all forms of salmon farming, regardless of how serious the risks from transgenic fish really are (Interview 1998.01; Interview 1998.25; Interview 1998.26; Interview 1998.30). Second, some salmon aquaculture advocates suggest that the release of transgenic fish might pose ecological risks different in kind or degree from those posed by selectively bred fish (Interview 1998.08; Interview 1999.21; Interview 1999.25). A British Columbia industry official distinguished the risks of selectively bred Atlantic salmon from the risks of their transgenic counterparts:

> [O]ver a hundred years and over 30 countries trying to stock [Atlantic salmon] in their rivers, there's never been one successful sustaining run of Atlantic salmon outside of their home range. And so we've got that kind of information. We don't know that with this [transgenic] thing. [T]he unknowns are just not worth the risk, particularly with something like this. You can do laboratory tests and you can put it with other species and things like that and see what happens. There's still going to be unknowns there. So I never see this being a part of net-pen or ocean-raised fish. Now if you can have completely escape-proof facilities, that's a different topic (Interview 1999.25).

According to this perception, the potential for unfamiliar ecological interactions because of novel life cycle traits makes escaped genetically engineered Atlantic salmon substantially riskier than their selectively bred counterparts.[iii]

The debate among scientists about potential environmental effects of transgenic fish contributes to an atmosphere of uncertainty and to the public's perception of environmental risk—a perception that ultimately may be more important than the details of agreement or disagreement among scientists. This interaction of uncertainty and perception is reflected in the report of the British Columbia Salmon Aquaculture Review:

> In view of the uncertain potential risks, and the serious public concern that has been expressed, it is recommended that the farming of transgenics continue to be prohibited in marine net-cage systems (Environmental Assessment Office 1997, v. 1, p. 81).

The environmental issues posed by escapes of transgenic fish into natural ecosystems are fundamentally questions about risk rather than certainty of harm. Proponents of transgenic fish suggest that too much is being made of potential harms that are unlikely to occur. For example, they argue that the residual risk from the escape of a small percentage of fertile transgenic fish would be no different than many other low probability events that our society considers to be acceptable risks. As described by a biotechnology company official, the issue is not elimination of risk but "risk management"—applying industrial, quality-control techniques to limit the risk:

> It could be completely auditable, like in any manufacturing process, because you're only going to say, 'Okay here's an agreed-upon sub-sample' (Interview 1999.41).

Other scientists argue that a statistically finite risk should not be acceptable when permanent environmental harm may result. As one molecular biologist put it:

> [A]t the moment, the only sure way that exists for containing them is to verify the triploid condition by flow cytometry of every animal, not: 'I'll test 30 out of this batch of 10,000, and yeah, they were all triploid.' That's not good enough because we just don't know. Unless one knows what risks are associated with that fertile individual, then I couldn't predict. So I would say 100 percent triploid is required, 100 percent sterile is required (Interview 1999.43, emphasis in original).

That is, complete reproductive containment is necessary because *any* release of fertile transgenic salmon could lead to irreversible ecological changes:

> There may be no going back here. [I]f you get one fish that has a sufficient fitness advantage that is not eliminated by stochastic variables it may take off (Interview 1999.43).

In the view of GE salmon proponents, there is no realistic risk that escaped transgenic fish will survive and reproduce. This lack of ecological fitness, they contend, reduces the need for proving 100 percent reproductive containment. One biotechnology company scientist argued:

> Are your fish more fit or less fit? If they're more fit, then some people would say, 'They're actually going to out-compete the fish out there.' Well, it's unlikely to be happening. The trouble is, if you quote the other side of the coin and say that they're now less fit, you know they're a problem, as all the ecologists say to you, 'They're going to interbreed and make the general population less fit.' So there's not a winning argument on this modeling. And it will take proper modeling with maybe some realistic data to come to some level of risk management here. If [there is] one fish in 10,000 or one fish in 100 thousand that's reproductively capable, what's its probability of actually making a match? Because survival is low in the wild (Interview 1999.41).

This clash of viewpoints about the potential risks posed by transgenic fish and the adequacy of triploidy as a reproductive containment measure is not likely to be resolved before regulatory decisions about deployment must be made. Recent research has suggested that traits of transgenic fish may simultaneously promote spread of the gene into a wild population and reduce viability of the offspring (Muir and Howard 1999).[ii] Because of the novelty of transgenic traits in natural ecosystems and our incomplete understanding of ecological relationships in aquatic systems, the environmental questions posed by transgenic fish are likely to remain sources of leverage for social resistance. Although scientific research and technical analysis will help frame the debate, decisions about uncertain risks are fundamentally the domain of political values and social agency, and cannot be resolved solely by science.

Observations about US federal regulatory provisions

I do not presume to suggest how Canada should proceed with its federal regulatory approach. It may be useful, however, to examine how the US regulatory system now operates, particularly the limitations of the US system.

The U.S. federal regulatory model does not currently include either substantive standards or transparent processes to address the ecological risks that underlie some social resistance. The federal Food and Drug Administration has taken the lead in conducting safety and environmental reviews for proposed use of GE Atlantic salmon. The agency bases its jurisdiction for both inquiries upon the transgenic manipulation of the fish's

growth hormone as a "new animal drug" under the federal Food, Drug and Cosmetic Act (Matheson 1999, 2000; Interview 1999.28). However, the FDA's enabling legislation contains no clear standard for environmental review. Consequently, the FDA is exploring new regulatory terrain at a time when public scrutiny is high and concern is increasing (Yoon 2000).

If the FDA approves use of GE salmon as food but imposes expensive restrictions on their deployment in the United States, the patent holder may sell its GE fish into another production venue such as Chile, which may return the finished food commodities to North American or other markets. That scenario is a nightmare for North American farmed salmon producers unless the domestic market turns against foods made from transgenic fish. Activist groups opposing this biotechnology are committed to neutralizing the value of FDA approval by raising consumer doubts, in the United States and in other major markets (Schurman and Munro 2003), about transgenic foods in general and genetically engineered fish in particular. If transgenic salmon receive FDA approval, the contest over this technology is not likely to end. For example, the social struggle will shift to local political battles within states and provinces that control tidelands and coastal waters. Because of aquatic organisms' mobility, enabling salmon to travel across national borders, there may be additional pressure for action under the Biosafety Protocol or other international agreements.

Limitations of current US environmental statutes

When regulation of ecological risks from escaped transgenic fish is considered, partial analogies can be found in federal environmental statutes designed either to limit discharges of pollutants (and certain chemical exposures) or to control introductions of exotic organisms. However, most environmental protection statutes require that harm be demonstrated empirically or experimentally to justify imposing regulatory constraints on private activities. These laws were not designed for precautionary control of exotic organisms, so they do not apply to risks that have not yet been manifested as harms or demonstrated in the laboratory.

Under the Clean Water Act (CWA, 33 U.S.C. §1251 et seq.), for example, opponents of salmon farms have successfully argued that escaped, non-native Atlantic salmon (not transgenic organisms in this case) are "biological materials" within the definition of "pollutant" in the act (33 U.S.C. §1362(6)) and require a National Pollutant Discharge Elimination System permit (see, e.g., *USPIRG v. Atlantic Salmon of Maine*, Civil No. 00-151-B-C, Recommended Decision of U.S. Magistrate Judge, February 2002, p. 12).[iii] Even if a discharge permit is required, the degree of protection afforded by the permit still depends upon the extent to which harm can be documented or substantial likelihood of harm can be established, and either of these evidentiary burdens will be difficult when the risk is uncertain (see *Marine Environmental Consortium v. Department of Ecology* (Pollution Control Hearings Board, 1998)).

In the absence of documented harm, statutory authorities for controlling pollutant discharges or chemical exposures usually require experimentally derived dose-response relationships. Within the limits of current scientific understanding, the risks from introductions of genetically engineered aquatic animals cannot be characterized in this way. In contrast, the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. §§136-136y) or the Toxic Substances Control Act (15 U.S.C. §§2601-2629), may apply to certain transgenic plants (such as Bt corn) in which the genetic modification operates to kill insects or to genetically engineered bacteria that produce chemical substances. However, transgenic fish do not fit within the coverage of these statutes without untenable distortion of either the statutory definitions or Congressional intent (see Saperstein 1990; Bisbee 1993; Gorski 1993; Kapuscinski and Hallerman 1994); see, e.g., 51 Fed. Reg. 23302, 23313, 23324-23325 (June 26, 1986), corrected by 51 FR 25412 (July 14, 1986)).

Nor do US federal laws that are intended to control introductions of harmful organisms provide adequate tools for dealing preventively with risks from transgenic fish. These statutes generally require evidence of damage, and they authorize control on the basis of a list of injurious organisms either designated by the statute or compiled by the regulatory agency as authorized by the statute (see, e.g., Lacey Act, as amended, 16 U.S.C. §§3371-3378). In the case of transgenic fish that have not yet been introduced, the requisite harm has not occurred, but if regulation is deferred until evidence of harm has been collected, the ecological impacts may be irreversible.

The US federal statute that comes closest to addressing prevention of harm to aquatic ecosystems is the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (16 U.S.C. §4701 and other codified sections). The act defines "aquatic nuisance" to encompass threats to "the diversity or abundance of native species or the ecological stability of infested waters" (16 U.S.C. §4702(1)). The rest of the statute, however, focuses primarily upon preventing harm from organisms introduced in ballast-water discharges from ships; and the legislation is simply too narrow to provide authority for precautionary control of risks from GE fish.

Current US regulation and the Coordinated Framework

Despite the absence of preventive authority under these federal statutes, US regulatory agencies have presumed that existing authorities for pre-market review are sufficient to provide protection for ecosystems. That presumption is explicit in the Coordinated Framework for Regulation of Biotechnology, formally adopted in 1986 by the President's Office of Science and Technology Policy and reinforced by that office's subsequent policy pronouncements. Federal agency review of biotechnology products must follow the procedural requirements of the Coordinated Framework (51 Fed. Reg. 23,302 (June 26, 1986)), which covers the regulatory policies of the FDA, Environmental Protection Agency (EPA), Occupational Safety and Health Administration, and Department of Agriculture (USDA), as well as the research policies of the National Institutes of Health,

National Science Foundation, EPA, and USDA.

Under the Coordinated Framework, genetically engineered products "will be reviewed by [the FDA, USDA, and EPA] in essentially the same manner for safety and efficacy as products obtained by other techniques" (p. 23304). Where more than one government entity may have authority to provide regulatory oversight, the Coordinated Framework establishes a lead agency and requires that all reviews be consolidated or coordinated through that agency. For transgenic Atlantic salmon, the FDA has the lead and is the only federal agency that will make a decision about whether these organisms may be produced and marketed.

Because growth acceleration in transgenic Atlantic salmon is accomplished through changes in hormone delivery, timing, and amount, the genetic manipulation is "intended to affect the structure or function" of the fish and satisfies the definition of "animal drug" in the Federal Food, Drug and Cosmetic Act (21 U.S.C. §§301–360bbb-2, 321(g), 321(v)). Consequently, the production and marketing of these fish requires pre-market approval by the FDA under the agency's regulations for new animal drugs (Matheson 2000; Interviews 1999.28 and 1999.02). In accordance with the Coordinated Framework, the U.S. Fish and Wildlife Service and the National Marine Fisheries Service—the federal agencies with the greatest expertise in aquatic ecology and in fisheries or wildlife sciences—participate only through the FDA's new animal drug evaluation.

In specific, limited circumstances, substantive provisions of the federal Endangered Species Act of 1973 (ESA) may apply (see ESA, as amended, 16 U.S.C. §§1531-1544). With respect to the ESA-listed Gulf of Maine Distinct Population Segment of Atlantic Salmon, for example, the U.S. Fish and Wildlife Service and National Marine Fisheries Service have requested interagency consultation under ESA Section 7(a)(2) on the FDA's consideration of Aqua Bounty Technologies' application seeking approval for its genetically engineered Atlantic salmon (Bartlett and Colligan 2001). However, for Pacific salmon population segments not listed as endangered or threatened, the ESA's statutory requirements for consultation do not apply.

Officials at the FDA have acknowledged that pre-market review of transgenic salmon presents the agency with "interesting challenges" through which the FDA's role in evaluating the potential environmental effects of transgenic organisms is evolving (Starke 2000, quoting Stephen Sundlof, head of the Center for Veterinary Medicine). In the words of another FDA official, "The agency is still struggling to find ways to make this fit the regulatory framework. [I]t's a bit of a round peg in a square hole. It's not a clean fit" (Interview 1999.02). The difficulties arise, in part, because no substantive standards exist, except the safety and efficacy determinations required for pre-market approval as a new animal drug, to guide the FDA's consideration of potential environmental impacts from introduction of these transgenic organisms (see 21 U.S.C. §§321(u), 355). The FDA's regulatory standards address the safety of the transgenic manipulation—how it

may affect the health of animals in which it operates or of human consumers who use those animals as food.

The FDA has indicated that its animal drug-safety standard is broad enough to include impacts on wild fish and other organisms in the environment that may be affected by introduction of transgenic salmon (CEQ/OSTP, 2001), but the statute explicitly addresses health impacts, not environmental harms or risks. Nor does the statutory context seem to contemplate ecological functions that might be changed by harm to wild populations. The National Research Council's Committee on Defining Science-Based Concerns Associated with Products of Animal Biotechnology (National Research Council 2002) has raised questions about how this asserted statutory authority would actually work (p. 112) and about whether the Federal Food, Drug and Cosmetic Act's safety standard for animal drugs is adequate "to sustain FDA's regulatory authority over broad, systemic effects of animal biotechnology on ecosystems" (p. 116).

Of the applicable statutory mandates, only the National Environmental Policy Act (NEPA, 42 U.S.C. §4321 et seq.) expressly requires the FDA to consider whether its decision would have significant environmental effects (see 42 U.S.C. §4332). However, NEPA contains no substantive standards; its requirements are entirely procedural. In addition, the FDA's regulations for implementing NEPA make the agency's environmental analysis much less accessible to the public than the processes followed for a typical NEPA environmental assessment or environmental impact statement. The FDA's environmental reviews of new animal drugs differ from most other federal-agency NEPA procedures because the FDA may actually reach a final decision to approve the product before the public receives information about the assessment of potential environmental impacts.

The regulations provide that, in order to protect trade secrets, many FDA investigations, reviews, and approvals of animal drugs are not disclosed. Therefore, the agency will not release the protected information, even if it is essential to the final decision. If the FDA determines that a proposed action may have a significant impact on the environment, an environmental impact statement will be prepared, but the analysis will be made available to the public "only at the time of the approval of the product" (21 Code of Federal Regulations [C.F.R.] §25.52). After the FDA has made its regulatory decision, the public may submit comments that "can form the basis for the agency to withdraw approval" (21 C.F.R. §25.52 (b)). Once the FDA has reached its decision on whether to approve the application, there is no mechanism for further federal agency consideration of the issue because, with the limited exceptions of the Endangered Species Act and certain authorities relevant to the siting of facilities, no other federal statute applies.

These secrecy requirements negate the usual public-disclosure function of NEPA procedures by precluding open discussion by scientists (including submission of supplemental information) and by nullifying the value of public comment that might shape the agency's determination during the environmental review. Instead, this approach

assigns to the public the burden of persuading the FDA to revoke an action it has already taken. Unless the agency delays the effective date of its approval, deployment of the product (e.g., transgenic fish) may proceed, thereby substantially undercutting the usefulness of the disclosure that forms the core of the NEPA process. Ironically, depending upon the approval conditions established by the FDA, deployment of transgenic salmon might already pose the risk about which the public may wish to comment. Under this approach to environmental impact assessment, transgenic fish may be deployed, and (depending upon conditions included in the FDA's approval) escaped fish may impose risks, before public comment has a realistic opportunity to affect the outcome.

Criticisms of the US approach from scientists

Some scientists and policy analysts have raised concerns about this approach. For example, the National Research Council (NRC 2002) has highlighted the risks from escape or inadvertent release of transgenic animals that could spread transgenes through reproduction with wild conspecifics, particularly species that easily become feral, are highly mobile, and have historically caused substantial community harm (p. 83). Animals with those characteristics include fish and shellfish. In some applications of biotechnology to animals, the NRC committee noted that "scientific uncertainty will be a particular concern" in the regulatory context because of the novelty of the issues and the lack of established methods for answering questions about the risks (p. 111). In addition to criticizing the "potential lack of clarity about regulatory responsibilities and data collection requirements" (p. 116), the committee expressed its "significant concern" about US federal agencies' legal and technical capacity in the environmental area (p. 118).

Final note: FDA Jurisdiction and GloFish™

As if to underscore the NRC's concerns about the adequacy of the legal framework as currently configured, the FDA declined to exercise its authority for pre-market review of genetically engineered zebrafish (*Danio rerio* [*Zebra danio*], GloFish™), which are distributed in the aquarium trade. In a press release that seems to depart from the agency's asserted basis for jurisdiction over genetically engineered physiological changes that constitute new animal drugs, the FDA (Food and Drug Administration, 2003) indicated that it would not review GloFish™ because they are not used as human food and therefore pose no threat to the food supply. In addition, the FDA news release stated, "There is no evidence that these genetically engineered zebra danio 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." The FDA provided no data or analysis to support its conclusion.

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D. Kelso Endnotes:

[i] Between June 1998 and September 2002, I collected data from industry and archival sources and conducted more than 100 field interviews in British Columbia, Washington, Maine, Alaska, California, and Washington, D.C./Maryland. Additional informal contacts with salmon industry representatives followed during 2003 and 2004. I also conducted legal research on U.S. federal regulatory policies during the period 1998-2004. Semi-structured interviews followed a question framework designed separately for each category of interviewees: salmon farm operators, government regulatory officials, biotechnology developers, research scientists, commercial fishers, salmon processors, hatchery managers, and nongovernmental advocacy organizations. Interview questions addressed technical problems and other production challenges, marketing issues, perceptions of risk to the environment and to the industry, social conflicts, and scientific uncertainties. To preserve anonymity, I identify the interviews only by year and serial number.

[ii] Environmental groups, fishermen, neighboring landowners, and some First Nations bands have raised criticisms ranging from pollutant discharges in the vicinities of pens to potential harms caused by escaped fish (see, e.g., Ellis and Associates 1996; Goldburg and Triplett 1997). Aquaculture industry advocates reject these charges and insist that opponents' concerns rest on conjecture rather than on scientific evidence showing actual impacts (see, *e.g.*, B.C. Salmon Farmers Association, undated-a and -b; Kenney, 1997). Introduction of ecologically competent "exotic" fish, whether Atlantic salmon in Pacific waters or transgenic fish with traits for which "there is not currently heritability" may pose substantial risks (Kapuscinski and others 1999, at p. 9).

[iii] Muir and Howard showed three theoretically possible outcomes. First, transgenic individuals may have much lower net fitness and gene flow that lead toward relatively rapid elimination of the transgene from the wild population. Second, transgenic individuals may have equal or higher net fitness than wild types and gene flow that lead

to the spread of transgenic genotypes throughout the wild population, an invasion hazard (Muir and Howard 2001). Third, transgenic individuals may have antagonistic trade-offs between fitness traits, leading to the "Trojan gene" effect (Muir and Howard, 1999), a population-extinction hazard that may arise from several combinations of trade-offs (Muir and Howard 2002).

In their work initially describing the "Trojan gene" effect, Muir and Howard (1999) observed one of these combinations when they released 60 transgenic Japanese medaka (*Oryzias latipes*) bearing extra growth hormone genes into a laboratory population of 60,000 fish of the same species. Deterministic equations applied to empirical life-history data in computer simulations showed that the larger size of transgenic males increased mating success, thereby spreading the transgene in the wild-type population; but the introduced transgene also reduced the viability of the offspring. Consequently, the experiment predicted that the transgene would drive the mixed population of transgenic and wild fish to half its abundance in less than six generations and eventually to local extinction. Subsequently, Muir and Howard (2001, 2002) refined their model and predicted that risks of invasion or extinction hazards could also result from other combinations of effects caused by "the pleiotropic action of transgenes on different fitness components of individuals" (2002: 103).

In another experiment, Robert Devlin and his colleagues (2001) injected a salmon gene construct "overexpressing growth hormone" into the eggs of two rainbow trout (*Oncorhynchus mykiss*) strains: one a relatively slow-growing wild fish and the other a fast-growing domesticated, artificially selected fish. The researchers observed that transgenesis can affect the final size of the transgenic wild-strain at sexual maturity ("an observation that warrants concern"), that both the transgenic domesticated and wild-strains had reduced viability, and that all the fish in the transgenic domesticated strain died before sexual maturation (p. 781).

[iv] The decision is accessible at http://www.med.uscourts.gov/Site/opinions/kravchuk/2001/MJK_08282001_1-00cv150_USPIRG_v_Heritage.pdf, and companion cases (Civil Nos. 00149-B-C and 00150-B-C); see also *Marine Environmental Consortium v. Department of Ecology* (Pollution Control Hearings Board, 1997).

Participant Discussion

Muir: A couple of comments referring to GloFish™. I don't think they ever applied to the FDA formally. They just marketed them and waited to see who would stop them.

Fletcher: No, Gong did the research and got all the approval he could before going ahead.

Kapuscinski: This was a departure for their (FDA) intent until now, and they've left wiggle room for the future. The FDA has never issued a formal policy (on regulation of transgenic fish). It doesn't look like they are going to waiver on their involvement in regulating environmental safety of transgenic fish.

Wright: On the GloFish™ web site they detail all of their attempts to approach different regulatory agencies. This is actually well documented. They had a number of experts provide analysis, including Dr. Muir. I think part of the issue was the official release of this was supposed to be in January, but they were marketed prior to that, and the FDA had to hurry to catch up. All of the analysis and market planning was only done in a couple of months.

Kelso: That could be an interesting point, my critique, was not of producers of GloFish™, but two very inconsistent positions of FDA over the environmental impact of these organisms. The FDA needs to be clear on the basis for exercising its authority. Whatever it is, it's not clear from this example. How can it be clear for a producer of a GMO fish if it is not clear to whom is in charge? I like the point you make on the way that this burst on the regulatory scene too late. One of the keys to better policy is to move decision and discussion upstream, away from this deployment decision.

Fletcher: I don't know why they got involved, when they decided that it's under the FDA. Environmental safety is part of the *Drug Act*; the problem is that it's not a great fit (this creates indecisiveness).

Kelso: Environmental safety is not in the FDA, but is an extension of the Act based on animal health. If you have to reach from animal health into ecology it is a difficult fit. Streamlining has gone too far in this case so there is a regulatory gap in assessing an animal's environmental or ecological impact.

Dunham: Perhaps FDA changes its approach case by case on how to do things, and on a day to day basis. They rely fully on Fish and Wildlife Services to handle the environmental issues. They might look like they are taking the lead, but they rely heavily on Fish and Wildlife Services.

Kelso: Although it appears that the FDA was taking the lead, the analysis will be done by one of the other official agencies that would have the expertise. That's an intriguing point. How do you get at that if that analysis remains secret until after the decision has been made?

Dunham: USFWS was saying that they had to be involved.

DISCUSSION SESSION 1

OVERVIEW AND DISCUSSION OF REGULATORY PROCESSES FOR TRANSGENIC FISH IN CANADA

George Arvanitakis

Presently, the *Canadian Environmental Protection Act, 1999* (CEPA1999) is the only Canadian federal government legislation mandated to conduct environmental and human health risk assessments of transgenic fish, and to propose risk management measures to minimize any such risks, were they identified.

The federal government definition of biotechnology is "the application of science and engineering in the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms."

Part 6 of CEPA provides the mandate for the notification and assessment of living organisms that are considered "new" (i.e., not on the Domestic Substance List (DSL)) substances. The New Substance Notification Regulations (NSNRs) are in place under the authority of CEPA. Part II.1 of the NSNRs deals with new substances that are living organisms. This Part includes provisions for the exemption from notification and assessment of living organisms undergoing research and development activities in contained facilities from which there is no release into the environment of the organism, its genetic material or material involved in toxicity.

Prior to the manufacture or import of living organisms, notifiers are required to submit information to Environment Canada and Health Canada which will form the basis of an evaluation of the potential environmental and human health risks of the organism. This information is outlined in Schedule XIX of the NSNRs: The information required includes details on taxonomy, genetic modifications, proposed uses, information and data on studies conducted to determine ecosystem and human health effects, contigency plans for accidental release, etc.

"Toxicity" is legally defined in CEPA itself. In practical terms, it means risk. Based on a thorough evaluation of the available information, should the organism be deemed "toxic" or suspected of being "toxic", then a number of control measures can be imposed. These can include Significant New Activity (SNAc) provisions, conditions and prohibitions.

After the assessment period is over, the notifier is also responsible for providing any new information that may become available that reasonably supports the conclusion that the organism is, or is capable of becoming, "toxic" as well as for correcting any information previously submitted.

The following web sites provide further information:

http://www.ec.gc.ca/substances/nsb/eng/reg_e.htm
http://www.ec.gc.ca/substances/nsb/bioguide/eng/bioguide_e.htm

Participant Discussion

Bughio: Health Canada, in collaboration with Environment Canada and DFO has prepared a Guidance Document for the CEPA, NSNR, and Schedule XIX for genetically engineered aquatic organisms. Currently, this document is undergoing internal review and will be distributed shortly for comments. Health Canada is the lead on this Guidance document. Also, the Government of Canada (Health Canada or Environment Canada) has not yet received any formal application for commercialization for transgenic Atlantic salmon or rainbow trout in Canada.

Maclean: One of the things in Europe is how relaxed regulations are about non modified foods. Is this also true in Canada?

Arvanitakis: CEPA does not just deal with GMOs, but also with any naturally-occuring, non-modified organisms manufactured or imported into Canada that may pose risks to human and ecosystem health. CEPA does not deal with human food safety. Health Canada's Health Products and Food Branch is responsible for conducting safety assessments on any foods derived from GMOs.

Maclean: What is a toxin as it is stated in CEPA?

Bughio: Bughio provided clarification and information on the: Current role and responsibilities of DFO in the regulation of transgenic aquatic organisms; Current status of DFO's Draft regulations; Clarifications on the role and responsibilities of CCAC; Current role and responsibilities of Health Canada/Environment Canada in the regulation of transgenic aquatic organisms; Role of Novel Food Directorate - Health Canada; Status of Guidance document for Schedule XIX requirements for transgenic aquatic organisms.; Industry is welcome to have a Prenotification Consultation with Environment Canada/Health Canada, if they require clarifications on the CEPA Schedule XIX requirements for their product.

Arvanitakis: Canada's regulations are a bit complicated as they presently stand. Peggy will talk more about DFO's present and future role in the regulation of transgenic fish. Hopefully we will have something more streamlined in the future.

Kapuscinski: I think that these issues have stimulated them (FDA) to think about traditional non-modified foods as well. Things are changing with the way existing Regulations are implemented. CFSAN has ability and power to regulate all foods. Overall assessment has been elevated.

Auer: In the EU we have two specific regulations that regulate seeds and transboundary movement of GMOs. Does CEPA rule the transboundry movement of GMOs?

Unknown: No, this is covered by one of the other acts listed under schedule four of CEPA 1999. For plants, the *Seeds Act* controls the movement of plants and the *Fisheries Act* controls the movement of aquatic organisms.

Poon: What is the role of the public health inspectors? Why is Environment Canada the only party responsible and Health Canada was not involved with enforcement?

Arvanitakis: EC has the legal mandate for enforcement under CEPA. At Health Canada we give them any technical information and assistance we can provide.

Poon: How about the provincial and municipal level.

Arvanitakis: You would have to ask the enforcement group at Environment Canada that. I believe that they would be in a better position to co-ordinate enforcement activities with provincial and municipal authorities if necessary.

Van Aggelen: GloFish™ were under full responsibility of Environment Canada under CEPA (in response to Poon's questions), not the Canadian *Food and Drugs Act.*

Bughio: GM fish are not regulated under any other Act in Canada, except CEPA. The intended use for the release of GloFish™ was not food, so it is not regulated under Health Canada's *Food and Drugs Act*.

Tsang: At this time DFO, under the Fisheries Act, has powerful legislation giving authority for the conservation and protection of fisheries resources. We have the power to make regulations, such as the fish health regulations and the national code on introductions and transfers. We are in the process now of developing regulations for GM fish. Currently there are draft regulations, but more work needs to be done. From the information we get today we can improve our thinking on the assessment framework. We need to make those regulations CEPA equivalent so there is consistent federal authority in the regulation of any GM animal. These regulations

will follow; a product-based approach instead of a process-based approach is being utilized. For now those regulations are for aquatic organisms with novel traits. This process will also involve the regulatory impact assessment. We also have draft policy on research and rearing of transgenic aquatic organisms. That draft policy is used to look after any activities at the research stage and requires inspection from regional authorities of DFO to ensure the facilities are up to code and contained. At this time while we are developing the regulations, we are still able to handle any applications that come in today and tomorrow because CEPA is there. DFO has an arrangement with Environment Canada and Health Canada to provide them with fishery expertise for assessment. We will administer the notification process and assessment and make the recommendations on the risk management options. Until we develop the regulations, this is the process we will follow. I think the CFIA is in the same situation in the terms of transgenic animals. They are working on regulations to make informed inspection decisions in terms of fish products entering Canada. As well, the definition of novel is being discussed so we can ourselves go through the process of policy and regulation making. CFIA prefers to go wide and non descriptive, so regulation needs to be done only once and covers all interested parties.

Arvanitakis: We have to look into the domestic substance list (DSL), and what we could put from the fisheries sector on that list. I believe we do have some information on that process.

Kochhar: CEPA is the over arching legislation. CFIA is a part based system, if something is intended for release, or has been released, then we are in control. What we have tried to do is team up with Environment Canada and Health Canada and provide them with expert advice on animal biotech specifically for farm and livestock animals. This can provide notifiers an idea of what kind of information we are looking for when applying. There needs to be clearer processes to identify the amount of information that is required and how that process will unfold. CFIA is at the front lines dealing with releases (for example, transgenic animals that got into the feed supply). We are trying to avoid these issues by creating appropriate guidelines.

Unknown: The *Fisheries Act* is quite powerful; we have extensive coordination between the federal government and the provinces.

Tsang: Under the framework of Introduction and Transfers, we issue licenses for any movements of organisms, especially if they are non-indigenous species.

Devlin: It is the regular routine to control movement of any fish within Canadian waters. Transgenic fish movements would thus also be controlled.

Fletcher: I like the CEPA regulations and it is obvious how you apply them. What I find with the FDA is there are no instructions and no way of knowing what to do. The web site is enormous. I like the Canadian approach because it gives very clear diagrams of what to do. Politics are involved in the core process too. Saves people money to have these clear guidelines.

Kapuscinski: Yesterday we talked briefly about the issue of transparency of regulatory processes, and I realize that under the regulatory duty, you have to protect confidential information, but can you give us a feel to the extent to which your assessment process now and into the future will allow public input into a particular application?

Tsang: At the very highest levels, the government is now processing smart regulations that make sense and are not burdensome. This is lead by the Privy Council office. DFO participates in some of the focus groups. It's a very high level and large process that the Governor General spoke about from the speech from the throne. Second point, the Canadian Biotech Advisory Council has made a recommendation in their report on GM foods on how federal regulation authorities are doing a fairly good job in regulating GM food.

Arvanitakis: One way in which we are dealing with transparency issues now, is through the process of preparing sanitized risk assessment reports for genetically modified organisms previously notified under CEPA. These risk assessment reports, are intended for posting on the Biosafety Clearing House of the Cartagena Protocol on Biosafety. In posting these reports on the BCH, we are acting as a model non-party, since Canada has yet to ratify the Protocol.

Kapuscinski: How are you going about the kind of process to decide what information should be on there as it's essential to the risk assessment? The core question is if a party claims confidential information very broadly, it may demand secrecy on an important part of an environmental assessment.

Arvanitakis: It is a fine balancing act. For example, we are presently negotiating with a previous notifier regarding what confidential business information contained in the risk assessment of one of their substances they may feel comfortable with posting to the BCH web site. We need to get more feedback on these issues from previous notifiers and balance their concerns with the information required for the Biosaefty Clearing House.

Kapuscinski: Is this issue something you will be addressing in terms of your formal deliberations in transgenic fish?

Tsang: I'm sure it will come up, and we will have to listen to views from everyone and we will come up with a decision. The public has certain expectations and the companies have certain demands on what they would want the system to do for them.

Lee: CBAC says that risk assessment should remain public except in regards to construct information.

Pare: Under CEPA schedule XIX, we have all of the information for GMOs. Guidelines are on the web site and they are preparing more guidelines, which are under internal review. You can contact us for consultation and we can provide all information to prepare your package.

Kapuscinski: Are these the new or draft guidelines?

Pare: This document provides information for each point on the NSNR. Ask enforcement that.

DISCUSSION SESSION 2

CURRENT AND FUTURE APPROACHES TO GENETIC CONTAINMENT AND TRANSGENE IDENTIFICATION

Lead: Tillmann Benfey

Benfey's lead Questions: What determines whether genetic containment is necessary? What determines the degree of stringency required for genetic containment? Who does monitoring?

Muir: I feel that employing different methodologies to assess net fitness and to estimate the likelihood that something could escape and is fertile and could reproduce is critical. Levels of containment decrease the chances exponentially. Then, the next level, if it does spread, is what is the degree of harm. It's a logical progression. One size doesn't fit all.

Kapuscinski: First of all there isn't one single answer, harken back to some of the points on biological confinement of GMOs. You want to know what the chances are of the spread of that organism. We have a fairly good capability to assess the status of the populations into which transgenes might spread. You may have some cases in which you can handle some moderate to low levels of stringency, and other cases where you need a high level of stringency, e.g., the coast of Maine where eight distinct populations of salmon are listed as endangered. Already the farmed fish are more abundant than the wild fish returning. In some cases the cages are right off the mouths of the rivers. It could be reasonable to say you need a high level of stringency in that situation. You can meet it several ways, e.g. combining monosex with triploid sterilization. Or you would need a higher screening level to remove failures (2N). You need information about the GMO itself and the status of the population it will directly effect. For Example, in Chile, with no endangered population of salmon (because salmon are not native there), one could assess whether the stringency could be lower.

Benfey: Key point there is that these should be assessed case by case.

Devlin: Uncertainty plays critical role in describing stringency.

Kelso: I'd like to point out that the issues often don't pertain specifically to safety. There are other concerns as well. Salmon have cultural weight and no matter how safe it is, they want the wild salmon protected against various impacts. The public doesn't want GM salmon to get out, in any

way. I think that we really need to look beyond the science. What are people thinking and what concerns them?

Fletcher: If we opt for triploidy for containment, and we know that it is not 100% effective and there is a small probability of release of transgenics into the environment, is this more or less harmful than the damage that has been done by current accidental releases of farmed salmon from net pens?

Kapuscinski: Farm salmon issues should also be addressed (concurring with Fletcher).

Fletcher: The issue in Maine is that the industry may shut down based on the stringent regulations that are going in to place.

Maclean: There is a moral discrepancy in Europe. Salmon farming is the most ecologically damaging aspect of European economy. The people involved in ecologically damaging industry are intricately involved in creating new legislation for GMOs in the same industry.

Devlin: Everyone comes to the table with a set of biases. The aquatic environment is viewed very differently than the way people view effects from food production from terrestrial environments from agriculture. There is a long history of damage to land ecosystems, so we are used to it, but the same is not the case for aquatic ecosystems.

Kelso: Regulatory systems protect reasonable expectations. Legal system and regulation system allows for change in response to new challenges. Built into it is a kind of gyroscope. Dr. Maclean's point is interesting. It comes with a manifestation of this kind of anomalous change and stability. It's difficult for regulatory agencies to accommodate that. One point Garth mentioned is if there is no greater risk from this new thing, (GMO's) than from conventional salmon aquaculture, does it make a difference (caution)? I would suggest that's not a good projection. It could be misunderstood. I think the point that is we have some obligation to engage how one takes a precautionary approach that isn't just an elegant way of saying no. It's a legitimate thing to say how we accommodate this tension between economic freedom and industrialism. That tension is to engage this reasonable expectation that society can try on different things without dispersing the risks to the public.

Devlin: Everything is balance between opportunity and risk. These are decisions that come out of analysis, and the social factors should be examined along with the economic and environmental issues. These issues are above current scientific risk assessments.

Kapuscinski: That's why it's helpful to aim for a risk decision process that provides room for scientific analysis and deliberations by officials, scientists, and interested parties. You can't have only the technical people decide what the benefits are going to be. Combine analysis and deliberations so that good quality scientific analysis informs the broader society regarding where the potentially affected parties want to come down with risk assessments.

Lead Question:

What are the most effective methods for physical containment? How reliable is all-female triploidy as a sterilization technique? What are the alternatives to triploidy?

Muir: Rather than alternative in question 3, should be augmentation rather that alternative. The failure rate is one out of ten thousand; on top of that you could apply monosex and interference. If you do both of those simultaneously, the risk becomes so small as to be acceptable.

Devlin: We're thinking sterility here, but whole other areas for containment can be accomplished. With those come specific public perceptions, and one thing we should be talking about is going out to see what the perception of viability or sterility containment approaches would be to the public.

Kapuscinski: First, what are the confinement options including disrupting sexual reproduction? Then how much stringency of confinement do we need for a particular case? In some cases you may not want to use triploidy; one of the drawbacks of inducing triploidy on GH fish is a drop in growth enhancement. If you designed another confinement method that doesn't have that cost in growth performance, what are the actual options? Then apply the approach of an integrated confinement system to determine how many combinations of confinement options to apply to meet the chosen stringency levels.

Dunham: Triploidy is nice option for salmon, but not for all species. If you have to have sterilization for these other species then we have to explore something else (other than triploidy induction).

Fletcher: Purely from a regulatory point of view, the minute you use a transgenic approach to introduce sterility it's difficult for a company to realize value. If you're looking at salmon you're looking at a lengthy period. Most companies are looking for things that need to get done in a short time. You have to think of something better than sterility. It takes you to the F_2 generation before you can even talk about it. It's difficult to capture value on a sterility technique.

Donaldson: In terms of methods other than sterility, where Atlantic salmon are grown, if both countries agree to produce all females, we automatically have containment without issues. On the east or west coast, you have to have the opposite type of salmon. Using all females can provide 100% containment. It's up to the government to decide if they want to implement these measures.

Kapuscinski: Dr. Donaldson's point just shows that we need to think about containment on a case by case basis.

Benfey's lead questions: What characteristics of the GMO determine whether monitoring is needed? What measures need to be taken to simplify transgene identification?

Kapuscinski: I am reluctant to say that "one size fits all" in tracking and monitoring different GMOs. If you have a case where no risk was concluded, maybe you would still want some level of monitoring. One still needs a process to make smart decision on stringency scaled by harm and risk of harm. What is the sampling protocol and what are the statistics we use to track their movement and what effect they had. A lot of thought needs to be put into long term monitoring programs. Are they feasible and economically possible? Maybe there will be some sort of cooperative approach

Kelso: Identification in what ever form it takes is desirable in the longer term to allow insurance to operate. If you can identify the sources if there is a problem in the future, you have command and control. Should a marker sequence approach be undertaken?

Devlin: Most known transgenes can themselves serve as marker sequences. A major difficulty could be determining what to do about detecting transgenes you don't know the molecular structure of? The effort for such identification approaches would come down to a balance of cost and probability of those things occurring in your food supply. I worry a little about unknown transgenes because there is a lot of research that gets done that isn't officially sanctioned or published. Can we assume that everything is done completely legally, and is reported?

Van Aggelen: I think ultimately it will be in the realm of the regulatory environment on how it shapes up on the omic needs to detect it. In the U.S or Canada, the need for marker sequences will be desirable to have some means of proving the technology is safe. The public will demand regulatory agencies to come up with a toolset.

Maclean: The transgene is the marker and there is no easy procedure where one could identify a GM if you do not know the construct used. The genome is dynamic and GM individuals will undergo translocation.

Dunham: If we don't know what we are looking for then it will be difficult to justify paying for things when we do know what to look for.

Cormier: Monitoring should be done in a way that is fit for purpose. From the Canadian Food Inspection Agency, I have a bias in terms of genomes intended for the food chain. The need for tracking and monitoring will be regular. For some animals that should be a consideration. Access to construct information and integration sites is important. I don't know any fish producer or broker that would like to hear he has thousands of pounds of fish stuck someplace.

Maclean: Tracking the transgene is difficult but not impossible.

DISCUSSION SESSION 3

RISK ASSESSMENT METHODOLOGY

Lead: Bill Muir

Muir: What approaches are suitable for risk assessment of GM fish? What is the best way to deal with uncertainty in data for risk assessments?

Dunham: Uncertainty is reality. The effects of a transgene depend very much on what variables you look at. For example, different species will have very different requirements for risk assessments (e.g., catfish, tilapia, or salmon). There is not going to be a standard approach across the board for all species or transgenes. Since we are talking about genotype X environment interactions, this could be the real key for designing experimental units. The greater the genetic difference and greater the environmental difference, the larger the GXE interaction.

Devlin: One of the keys for risk assessment is to design a experimental facility that can within reason mimic the natural environment as closely as possible where fitness can be examined and GXE effects can be studied. This is particularly true for species that come from large complex ecosystems like salmon. GXE effects are critical both for experimental protocols and for influencing the phenotypic characteristics of fish used in experiments. The issue is to understand how different environments that we raise fish in produce particular phenotypes and how do these affect the reliability of our data. Culture conditions can dramatically affect the expression of a phenotype such as growth or spawning ability compared to the same genotype derived from nature, making studies with culture-reared transgenic fish questionable for risk assessments as we do not know the phenotype of this genotype in nature due to GXE uncertainties. We would love to have our laboratory equivalent to nature, but currently we only have facilities that can mimic environments for early life history stages with some reality. Marine environments present a very large problem.

Muir: In one extreme you could put the GM and wild type under the most optimal condition in the lab. If the GM fish can't compete even in the most favourable lab environment, I don't think it's possible it would survive at all in nature and is not a risk.

Devlin: What we find is that sometimes the best conditions in lab facilities can result in conflicting fitness characteristics (i.e., great fitness in competitive feeding behavior, but reduced mating performance.) This makes determinations of net fitness more difficult as we do not know the true

magnitude of the fitness effects that would exist in a more complex natural habitat.

Kapuscinski: For species like salmon they are a challenge, they go both through fresh and sea water. I think Bob's right, we need one place in the world where there is a really adequate facility that is a reasonable microcosm that is a surrogate to the natural environment. What if we got all the countries in the world to agree on building a very good environmental research facility. If there was one good place, then we could get all the smart interested people to work collaboratively. Also, another place in the world for both warm and cold water fish. We could move a lot faster in this area.

Donaldson: Salmon who were feeding on pellets and then were released into the ocean were subsequently found with little pieces of seaweed in their stomachs that looked like salmon pellets. It is thus important to use live feed in these studies.

Devlin: Fitness estimates could also be obtained from the release of verified sterile males into the environment, but this is probably not a wise scientific approach at this time given the uncertainties of risk with transgenics. Using surrogate systems like implanting fish with slow release growth hormone could also be useful, as Jorgen has shown, but this is not allowed in Canada.

DISCUSSION SESSION 4

TRAITS LIKELY TO BE MODIFIED IN THE FUTURE; BENEFITS (ECONOMIC AND SOCIAL); POTENTIAL RISKS TO THE ENVIRONMENT

Lead: Garth Fletcher

Fletcher: What traits do we see coming in the near future? Will DNA vaccines be treated the same way as GMOs? What we need are suggestions from everyone else. One idea is animals that can convert omega-6 to omega-3's. This is of course in the pipeline.

Devlin: Environmental tolerance changes, production enhancement, (growth, disease resistance) and nutritional quality are really the three biggest issues. Take omega-3 production for example; can we perceive that change to pose any environmental risk? This may depend on whether the organism is a critical prey item in nature, and if the particular ecological relationship of the predator requires omega-6 fats in the natural environment. Each transgene will need to be looked at on a case-by-case basis.

Kapuscinski: We shouldn't forget that there are some other aquaculture traits of high interest to producers; disease resistance is one of the ones likely to come up soon. It's hard to really predict, but it's reasonable to think that in the next 5-10 years there will be efforts to genetically engineer other traits of importance to aquaculture. Another point is, we are not talking about only finfish. I wanted to ask also – are there any discussions or interest in Canada of the new idea of purposely engineering a deleterious gene into an invasive species for biological control. The Australians are very interested in this for invasive carp. They have been doing some in-depth work to put into place the scientific aspects of risk assessment. They also have already held one major public deliberation workshop. The workshop involved even the people who presently earn an income from the commercial harvest of invasive carp. Their approach is a good model on how you can do this analytic risk assessment up front without stopping the research. Has there been any talk about that kind of application of genetic engineering in Canada?

Masri: In forestry, they are trying to develop viruses that acts on the invasive species and they are pushing that right now. There are companies in the United States that have applied to sell these viruses.

Kapuscinski: Part of why I raised the case of using transgenic fish for biological control of invasive species, is because it's a different ball of wax for doing risk assessment. You don't want to confine such a GMO because it's meant for release and spread into the populations of the invasive species. There are some risk issues that need to be addressed. If I was in the shoes of the regulatory agency, I would develop policy and regulations that are generic enough to be applied to this kind of case. So, biosafety regulations should address both sides of the coin: cases in which the transgene should remain confined to the farmed line of fish and cases in which the goal is to purposefully spread the transgene to an invasive or pest population.

Benfey: Two examples that follow the model are in the great lakes – sea lamprey and zebra mussels.

Lee: I think in Canada in forestry they are doing that against the pine beetle. They are producing a virus that attacks the beetle.

Fletcher: Sterility with transgenics is one of the emerging and essential research topics. What we need are ideas, and people that are making the regulations need to know what issues to be looking for.

Muir: For the whole range of new aquaculture products, 99.9% were covered in Bob's broad categories. One other might be medical models, e.g., dystrophic pig as a model of human health. We are developing pigs as a better model for humans than fish.

Kapuscinski: You cannot predict applications but you can look at what is in the pipeline. Clearly the regulatory system is going to need to be enhanced to look at other traits other than growth enhancement. There is going to be some work in commercial companies for medical models and veterinary health, and regulations need to be flexible enough to deal with these three. If in a few years, there will be disease resistant transgenic fish, the developers of these transgenic fish may be as frustrated as Garth is right now.

Maclean: What if Atlantic salmon were capable of creating prions. There is not currently a gene knock out, but this might be an interesting area to explore.

Dunham: The other classification is transgenic zebra fish altered to study different physiological pathways. Even these types of organisms, not intended for research, will be regulated.

Wright: That falls under CEPA. But it gets a little grey because it's a fish. CEPA is very clear about rats and mice that are GE. As long as they are not to be

released into the environment, there doesn't need to be notification. Probably 95% of all genetic modifications of fish are for research and not intended for release. They should be treated as rats and mice as long as they are laboratory animals not meant for the environment.

Wright: CCAC has decided how to handle fish. One of the official proclamations is to notify DFO.

Bughio: CCAC is a private organization. CCAC does not regulate transgenic organisms. If you are working with GMOs in general, no matter what organism, according to Section 29.16 of the New Substances Notification Regulations of CEPA, new organisms are not subject to CEPA regulations if they are research and development substances that are manufactured or imported in Canada such that there is no release of the living organism, its genetic material, or any material from the organism involved in toxicity, into the environment But if this research and development organism, its genetic material, or any material from the organism is released intentionally or accidentally released into the environment then you have to notify under CEPA.

Wright: Just trying to make sure that the idea is applied to everything. There is now going to be a DFO document, and that is what I am concerned about.

Bughio: It will be some time before DFO gets the authority to regulate transgenic fish. So for now, the transgenic fish are regulated under CEPA. Information on CEPA is available on the web site, and we are approachable. Please contact us if you have any questions. Industry is welcome to have a prenotification consultation with Environment Canada/Health Canada, if they require clarifications on the CEPA or NSNR Schedule XIX requirements for their product.

Devlin: There is some ambiguity in that section of NSNR. Organisms are shedding cells and DNA all the time, so if we take Section 29.16 literally, then this is a requirement that almost no facility can meet. The regulations should say that you should not allow viable germ cells into the environment.

Bughio: When CEPA was developed it was not intended for GM organisms. The people who were really drafting these things didn't anticipate what was coming their way.

Lee: We always have the option of having prenotification consultation for any proponents of these types of products so they know what is expected of them.

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