STATUS OF GENETICALLY MODIFIED (TRANSGENIC) FISH: RESEARCH AND APPLICATION

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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. Cloned populations have been produced via gynogenesis and androgenesis (Dunham 2004 in press), but direct cloning of an individual fish of interest has not yet been accomplished. This review will focus upon transgenic fish.

Techniques to produce transgenic fish

Gene transfer research with fish began in the mid 1980's utilizing microinjection (Zhu et al 1985, Chourrout et al. 1986, Dunham et al 1987). Zhu et al. (1985) published the first report of transgenes microinjected into the fertilized eggs of goldfish. In almost all fish gene transfer research, the foreign gene was microinjected into the cytoplasm of one-to- four cell embryos (Hayat 1989) as pronuclei are extremely difficult to visualize in live one-cell fish embryos. Ozato et al. (1986) used a slightly different approach, and injected the oocytes of the medaka, which had been removed from the ovaries nine hours before ovulation. The chicken delta-crystallin gene was injected and found in four of the eight medaka embryos examined (Ozato et al. 1986). An average of about 5% of the surviving microinjected embryos integrate the foreign DNA.

Microinjection is a tedious and slow procedure (Powers et al. 1992) and can result in high egg mortality (Dunham et al. 1987). After the initial development of microinjection, new techniques such as electroporation, retroviral integration, liposomal-reverse-phase-evaporation, sperm-mediated transfer and high velocity microprojectile bombardment were developed (Chen and Powers 1990) that sometimes can more efficiently produce large quantities of transgenic individuals in a shorter time period.

Electroporation involves placing the eggs in a buffer solution containing DNA and applying short electrical pulses to theoretically create a transient openings of the cell membrane, allowing the transfer of genetic material from solution into the cell. The efficiency of the electroporation is affected by a variety of factors including voltage, number of pulses and frequency of pulses.

The first successful gene transfer utilizing electroporation produced integration rates and survival similar to that for microinjection (Inoue et al.1990). Powers et al. (1992) then demonstrated that electroporation can be more efficient than microinjection with integration rates sometimes as high as 30-100%. Walker (1993) found that hatching rates were higher for electroporated embryos than for microinjected channel catfish embryos, and post-fertilization electroporation treatments had higher hatching rates than electroporation of sperm and then eggs prior to fertilization.

Efficiency of gene transfer is determined by several factors including: hatching percentage, gene integration frequency, the number of eggs which can be manipulated in a given amount of time and the quantity of effort required to manipulate the embryos. In this regard electroporation is a powerful technique for mass production of transgenic fish.

Retroviral vectors containing the envelope protein of vesicular stomatitis virus have been developed (Burns et al. 1994), and used to produce transgenic fish (Lin et al. 1994, Yee et al. 1994, Lu et al. 1996). Integration rates may be increased because of active infection. Unfortunately, these vectors are prone to unstable expression or even complete silencing of

transgene expression. Sarmasik et al. (2001) successfully utilized retroviral constructs to produce transgenic crayfish and topminnows, *Poeciliposis lucida*. The pantropic retroviral vectors were derived from the hepatitus B virus and the vesicular stomatitis virus, a pathogen similar to hoof and mouth disease which infects mammals, insects and possibly plants. The vector sticks to most cell membranes of any species. Transgenic crayfish and topminnows were produced by injecting immature gonads with a solution of the vector about one month before the normal age of first reproduction. Matured injected individuals were mated with normal individuals, and produced 50% transgenic offspring. Integration, expression and transmission of the pantropic retroviral - reporter transgene were observed for at least three generations. This is a very good gene transfer technique for live-bearers and fish, in general, but, introduction of viral sequences, into food fish may not be accepted by the public.Use of transposases to enhance integration rates may be a more viable option than retroviral vectors for oviparous aquatic organisms, but does not solve the problem of live-bearers.

Theoretically, inactivation of a gene can be accomplished by knockout of the gene by replacing the original gene with a mutated copy of the gene, or by disruption of gene expression using the antisense approach or the ribozyme technology. Although the later two approaches are currently feasible, the knockout approach is the ultimate method for gene inactivation because it will eliminate the gene products completely. Another technique for post-transcriptional gene silencing is utilization of RNA antisense constructs. Both double stranded RNA and antisense RNA were effective in disrupting the expression of GFP in transgenic zebrafish (Gong et al. 2002). Various antisense technologies appears feasible (Dunham 2004).

Regardless of the method of transfer, the foreign DNA introduced to the developing embryo, it appears to initially replicate and amplify rapidly in the cytoplasm of the developing embryo, and then disappears as development proceeds (Houdebine and Chourrout 1991). Integration at the one-cell stage has never been observed, thus the delayed integration causes mosaicism, (Stuart et al. 1988, 1990, Culp et al. 1991, Hayat et al. 1991, Gross et al. 1992, Hackett 1993) and, not all tissues contain the transgene and not all cells within the transgenic tissues harbor the transgene.

Copy numbers can range from one to several thousand at a single locus, and, in contrast to the head-to-tail organization observed in the mouse system, in some but not all cases the DNA can also be found organized in all possible concatemeric forms (Tewari et al. 1992), suggesting random end-to-end ligation of the injected DNA prior to integration. Transgenes can integrate at single or multiple chromosomal locations for individual transgenic fish.

For salmonids, the frequency of transgene transmission from founder animals averages about 15%, suggesting that integration of the foreign DNA occurs on average at the two-to-four cell stage of development (Devlin 1997b). Transmission of transgenes to F2 or later progeny occurs at Mendelian frequencies (Shears et al. 1991), indicating that the DNA is stably integrated into the host genome and passes normally through the germline.

Species of interest

Transgenic fish have been produced for numerous species of fish including non-commercial model species such as the loach, *Misgurnus anguilIicaudtis* (Maclean et al. 1987a), medaka, *Oryzias latipes* (Ozato et al. 1986), topminnows and zebrafish, although Gong et al. (2002) have developed transgenic rainbow zebrafish for the ornamental fish industry. Several experiments have evaluated transgenic farmed fish species including goldfish (Zhu et al. 1985), common carp ,silver carp, mud loach, rainbow trout (Chourrout et al. 1986), Atlantic salmon, coho salmon, chinook salmon, channel catfish (Dunham et al. 1987) and Nile tilapia (Brem et al. 1988). Additionally, gene transfer has been accomplished in a game fish, northern pike (Gross et al. 1992).

Results of gene transfer experiments

Reporter genes Reporter genes have been utilized to develop gene transfer technology, to facilitate screening of transgenic individuals and to study gene expression. Transgenic fish containing bacterial genes, b-galactosidase (McEvoy et al. 1988), neomycin resistance, goldfish (Yoon et al. 1989), hygromycin resistance (Stuart et al. 1988) and chloramphenicol transacetylase (tilapia) (Indiq and Moav 1988) have been utilized to examine various research issues. Currently (Amsterdam et al. 1995, Gong et al. 2002) green fluorescent protein gene and other fluorescent pigmentation genes are widely studied to effectively study development and gene expression.

Performance of transgenic fish-Growth

Positive biological effects have been obtained by transferring transgenes to fish in some, but not all cases, and the greatest amount of work has focussed on transfer of growth hormone 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 examined (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. 1989ab, Penman et al. 1991), despite the fact that salmonids are very responsive to growth stimulation by exogenously administered mammalian GH protein (McLean and Donaldson 1993). 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. 1992a, 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 grew 10-80% faster than non-transgenic fish in aquaculture conditions.

Subsequent experiments demonstrated that growth can be enhanced through transgenesis from 10% up to an incredible 30- fold. Du et al. (1992) used an all-fish GH gene construct to make transgenic Atlantic salmon, and report 2- to 6-fold increase of the transgenic fish growth rate. *Oreochromis niloticus* possessing one copy of a eel (ocean) pout promoter-chinook salmon growth hormone fusion grew 2.5-4 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. Preliminary results indicated that homozygous transgenic Nile tilapia produced from the ocean pout antifreeze /chinook salmon GH construct have growth similar to that of the hemizygous transgenics. Insertion of other GH constructs into tilapia have also yielded positive results, but not as dramatic as those with the salmon GH constructs. Two possible explanations for the difference in results are the type of construct and the type of tilapia studied was different. Introduction of a CMV/tilapia GH construct into a hybrid *Oreochromis hornorum* resulted in a 60-80% growth acceleration (Martinez et al. 1996, Estrada et al. 1999) depending on the culture conditions.

When introduced into coho salmon, cutthroat trout, *O.clarki*, rainbow trout, and chinook salmon, GH gene constructs 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 elevated circulating GH levels by as much as 40 fold (Devlin et al. 1994b, Devlin 1997b), resulting in up to five-to-thirty-fold increase in weight after one year of growth (Du et al 1992, Devlin et al. 1994b, 1995ab,Devlin et al. 2001), and allowing precocious development of physiological capabilities necessary for marine survival (smoltification). The largest of these P1 transgenics were mated and produced offspring with extraordinary growth. 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. .

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 3-6 fold accelerated growth rate compared to non-transgenic salmon (Du et al.1992, Cook et al. 2000a). Insertion of sockeye MT-B-sockeyeGHcDNA1 (Devlin 1997b) produced a similar result, 5-fold growth enhancement. Varying results among species and families might be related to different gene constructs, coding regions, chromosome positions and copy numbers.

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 rated 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.

Domestication is also important in transgenic growth responses, and Devlin et al. (2001) first observed that salmonid GH gene constructs that had a dramatic effect on growth in wild rainbow trout strains (with naturally low growth rates) but little or no effect in strains where growth rate has been enhanced by selection. In comparison, GH transgenic channel catfish derived from domesticated and selectively-bred strains exhibit only a moderate growth enhancement (41%). However, additional data on transgenic rainbow trout (Devlin et al. 2001) refutes this hypothesis on 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. When F77 was crossbred with a 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, 18X larger than the non-transgenic wild parent, 13X larger than the non-transgenic wild X domestic crossbreed, 9X larger than the non-transgenic domestic parent and more than 2.5X 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.A transgenic with 50% of its heritage from domestic sources was much larger than a wild transgenic, so great response from some domestic genotypes is possible.

Cold Tolerance-Most efforts in transgenic fish have been devoted to growth enhancement, although there are reports of improvement in cold resistance (Fletcher and Davies 1991, Shears et al. 1991). Early research also involved the transfer of the antifreeze protein gene of the winter flounder (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 of salmon. However, preliminary results with goldfish show some promise for increasing survival within the normal cold temperature range.

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 classification of pathogens. Bacterial disease resistance may be improved up to 3-4 fold through gene transfer. Insertion of lytic peptide cecropin B construct enhanced resistance to bacterial diseases 2-4 fold in channel catfish (Dunham et al. 2002e). There was no pleiotropic affect 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 (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 fish family carrying preprocecropin B and two families with porcine cecropin P1 , 0-10% cumulative mortality 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.

Pleiotropic effects-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 regulatory sequences exhibited about 3.6X less food consumption than nontransgenic controls, and food conversion efficiency was 290% 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.

GH transgenic Atlantic and coho salmon had intestinal surface area 2.2 times that of control salmon and the growth rate was about twice that of controls (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.

The insertion of the rtGH gene altered 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. Remaining transgenic individuals had higher survival than controls when subjected to a series of stressors and pathogens such as low oxygen, anchor worms, *Lernia*, *Aeromonas* and dropsy. 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, 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 had a lower ventilation rate when subjected to low dissolved oxygen compared to controls.

GH transgenic fish also exhibit body composition changes, but they are not as dramatic as those observed in mammals. Moisture content in GH transgenic Atlantic salmon was higher, relative to protein and ash, than in normal controls (Cook et al. 2000a). Chatakondi et al. (2002) 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 (an increase in 7.5% protein and a 13% decrease in fat). Moisture was lower in

F1 transgenic muscle, but unchanged in F2 transgenic individuals. 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 *O. hornorum urolepis* containing the tilapia growth hormone (tiGH) cDNA 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. 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 also had increased numbers of mitochondria in the cell, increased numbers of glycogen globules, increased numbers of muscle fibers, but reduced number of fat globules. 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 of transgenic catfish flesh was slightly better than non-transgenic controls (Dunham and Liu 2002.). 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 somitic muscle were affected, suggesting that that the transgenic salmon grew 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.

Zhu (1992) reported an increase in muscle thickness and body width in transgenic common carp, containing the human growth hormone gene. 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 were 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 most families of transgenic common carp (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 which is similar to the result for transgenic salmon because length changed more rapidly than weight in transgenic salmon (Devlin et al. 2001). 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. 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. The altered body shape of transgenic common carp resulted in improved dressing percentage in the F2 generation, and a similar result was obtained for transgenic channel catfish containing the same GH construct.

Excessive levels of growth hormone resulted in morphological abnormalities in the head, fin, jaw and operculum as a result of excessive cartilage and bone growth of the fastest growing

transgenic salmon (Devlin et al. 1995a). Insertion of a pOnMTGH1 gene construct into coho salmon altered centroid size (Ostenfeld et al. 1998). The dorsal caudal peduncle and abdominal regions were also distinctly enhanced in transgenic fish when compared to controls Morphological changes of both whole body and syncranium were prominent.

GH gene transgenesis also affects gill morphology as 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 two species. Pacific transgenic 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.

Progeny of salmon that grow 30X normal 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), analogous to the mammalian acromegaly syndrome. This effect can be sufficiently severe such 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 (10X) stimulated (Devlin et al. 1995ab). 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. 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).

Reproductive traits have not been greatly affected by GH transgenesis. Fecundity is not affected by insertion of rainbow trout GH cDNA 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. Transgenic rainbow trout experienced early maturation at 2 years of age, but in the same season as the controls.

Color changes in the GH transgenic coho salmon (Devlin et al. 1995b, Devlin 1997b). Individual containing opAFP or OnMT salmon GH contructs have lighter skin pigmentation and 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 enhance silver coloration and osmoregulatory ability (Devlin 1997b).

Combining genetic enhancement programs- In Israel, Hinits and Moav (1999) were able to improve common carp growth more by using genetic engineering with crossbreeding more than by using crossbreeding alone. Similarly, when salmon metallothionein promoter/salmon GH1 cDNA, OnMTGH1, was transferred to another wild rainbow trout strain, F77, growth was enhanced 7-fold which was almost 4-fold more than a domestic rainbow trout (Devlin et al. 2001). However, the transgenic wild X domestic crossbreed was by far the largest genotype, 18X larger than the non-transgenic wild parent, 13X larger than the non-transgenic wild X domestic

crossbreed, 9X larger than the non-transgenic domestic parent and more than 2.5X 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.

Genotype X environment interactions- Genotype-environment interactions occur for growth of transgenic channel catfish (Dunham et al. 1995) containing salmonid growth hormone genes, and the transgenics 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 as is the case for most genetic improvement programs, genetically engineered fish need adequate food to express their potential.

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

Several models have been developed that estimate and indicate genetic risk of transgenic fish. Muir and Howard (1999) evaluated a model and termed the Trojan gene effect, the extinction of a population due to mating preferences for large transgenic males with reduced fitness. Their conclusion is that both 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 a transgene has a male-mating advantage and a general viability disadvantage, analogous to the Trojan gene effect of Muir and Howard (1999). Hedrick's results indicate that 66.7% of the possible mating combinations for possible mating and viability parameters for the transgenes invasion into a natural population, the transgene increases in frequency, and for 50% of the combinations, 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.

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's (2001) interpretation was 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 defect in the simulation was the fact that the effect of predation in the wild could not be included in the model, biasing viability estimates (Muir and Howard 2001).

Although these modeling experiments based on laboratory data on small model species illustrate potential risk of transgenic fish, some weakness exist. The environment was artificial,

the mating preference does not exist for many fish including catfish, the models do not account for genotype-environment interactions which are likely, predation is absent as Muir and Howard (2001) indicate and the overall performance of the fish is not accounted for.

Risk of transgenic fish should be similar to risk of domestic fish and 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 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 sheer force of numbers. Transgenic fish would make an impact in this scenario, but again the consequences should not vary much from that of fish genetically altered by other means.

The reproductive performance, foraging ability, swimming ability and predator avoidance are the key factors determining fitness of transgenic fish, and should be a standard measurement prior to commercial application. All available data indicates that transgenic fish are less fit than non-transgenic fish, and would likely have little if any environmental impact. Extremely fast growing salmon and loach have low fitness and die (Devlin et al. 1994b, 1995ab).

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.

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. 1992a, 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). 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. This lower survival of the transgenics may be due to starvation. Transgenic Atlantic salmon fish had higher metabolic rates, and lost protein, dry matter, lipid and energy more quickly than controls (Cook et al. 2000a).

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.

Silversides, *Menidia menidia*, from Nova Scotia ate more food, had more efficient feed conversion and grew faster than a population from South Carolina (Billerbeck et al. 2001a), however, the 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. 2001b). Maximizing energy intake and growth rate engenders 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. 2000bc), 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) indicates 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 was the change in the syncranium and the head of the transgenic s 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 (250g) containing a gene 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, the transgenic fish consumed 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. This aggressiveness in seeking food likely is a factor for increasing vulnerability to predation.

All transgenic fish evaluated to date have fitness traits that are either the same or weaker compared to controls. The increased vulnerability to predators, reduced swimming ability, 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.

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. 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 2004). Additionally, fertile brood stock are necessary, so risk is minimized but not eliminated. Transgenic sterilization is potentially a much better option than triploidy.

Transgenic sterilization- Carp beta actin-tilapia salmon type GnRH antisense construct was injected into Nile tilapia (Norman Maclean, personal communication). 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 seabream GnRH antisense construct was injected into Nile tilapia, but 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 seabream and salmon GnRH antisense transgenics gave hatch rates that appeared to be dictated by the salmon GnRH antisense parent.

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

Another strategy, introduction of "Sterile Feral " constructs, disrupts embryonic development, thus sterilizing brood stock. Preliminary results show promise for this approach (Thresher et al. 2001).

Food safety of transgenic aquatic organisms

The general public has little understanding of biology and the vagaries of how their food is grown and where it comes from, so public education of the positive and negative aspects of transgenic food and its risks is lacking and is needed (FAO 2001). Food safety issues posed by transgenic fish are discussed by Berkowitz and Kryspin-Sorensen (1994). Concerns have been voiced of the possible risks of consumption of transgenes, their resulting protein, potential production of toxins by aquatic transgenic organisms, changes in the nutritional composition of foods, activation of viral sequences and allergenicity of transgenic products. These risks have been analyzed, and while the majority of genetic modifications to foodstuffs will be safe the greatest potential for risk and harm is allergenicity.

A transgenic soybean has been developed expressing a gene from Brazil nut to increase its protein content and this transgenic soybean was allergenic to some humans (Nordlee et al. 1996). The potential allergic reactions to transgenic proteins from the donor organisms is one of the strongest arguments for the enactment of some type of labeling (Hallerman 1992,1993,2001, FAO 2001).

In the case of the transgenic GH fish, specific experimental evidence that teleost GH is not active in primates was obtained by Guillen et al. (1999). Juvenile monkeys, *Macaca fascicularis* , (macques) were injected with 1,000 ng/kg of recombinant tilapia growth hormone per day for 30 days, equivalent to administering 70,000 ng/day to a 70 kg human. Blood parameters examined included hemoglobin, serum total proteins, blood glucose, packed cell column, total leukocytes and total erythrocytes. Body weight, rectal temperature, heart rate and respiratory rate were recorded daily. Head to tail length, interscapular cutaneous pleat, left-flank cutaneous pleat, cranial circumference and cranial diameter were measured. . Tilapia GH did not affect animal behavior pattern or food intake. Body weight, temperature, heart rate and respiratory rate were unaffected by tilapia GH administration to macques. The blood profiles and somatic growth of tilapia GH treated macques and controls were not different. Autopsies revealed all organs, tissues and cavities were normal, and no changes relative to controls were detected for common targets of GH such as tongue, palate plate concavity, liver, muscle, heart, kidneys and others.

Subcutaneous and abdominal fat, mesenteric fat and peritoneal fat were unchanged, normal and of usual color. No histopathological or morphological changes were observed.

Twenty-two humans were fed tilapia (transgenic hybrid *Oreochromis hornorum)* that contained and expressed tilapia GH transgene (Guillen et al. 1999). These tilapia grew twice as fast as non-transgenic controls. The humans were fed transgenic or control tilapia for 5 consecutive days, twice daily. Hemoglobin, total serum proteins, glucose, creatinine, cholesterol, leukocytes and erythrocytes were measured. No clinical or biochemical parameters and no blood profiles of humans evaluated before and after onset of experimentation were affected by consuming transgenic tilapia.

The fact that tilapia (teleost GH) did not promote modifications of blood glucose values, total protein and creatinine as well as having no affect on growth, target tissues, lipolysis, protein synthesis in the muscle or any contra-insulin effects is indicative and confirms that fish GH is not bioactive in primates. GH should stimulate erythropoiesis and lymphopoiesis and increase spleen and kidney weight (Gluckman et al. 1991) and is associated with stimulating fluid retention, growth, changes in blood volume and blood characteristics (Ho and Kelly 1991), but none of these phenomenon were observed.

Additionally, (Dunham 2004) analyzed the theoretical food safety of GH transgenic salmon. Levels of GH and IGF expressed by transgenic GH salmon are not always outside the range or much greater than the upper limit of GH and IGF secretion for other fish, food animals or humans, salmon GH and IGF are not bioavailable when orally ingested (cooked or raw, adequate cooking would denature the proteins), even if they were totally bioavailable the dose from one meal would only be a small fraction of total, daily, human production of GH and IGF, growth hormone is not orally active in higher species, sGH is not bioactive in humans, the primate growth hormone receptor binds only primate growth hormone and it requires both binding sites to be occupied, initial studies indicate fish GH has no biological effect on primates and short-term ingestion of GH transgenic fish has no biological effect on humans. The lack of oral activity of GH and IGF-I and the nontoxic nature of the residues of these compounds, even at exaggerated doses, demonstrates that salmonid GH and IGF-I present no human safety concern when consumed orally. Thus, viewed from a number of aspects, any increased concentrations of GH or IGF in edible salmon skeletal muscle or skin is not hazardous to human health.

Commercial application

Commercialization of transgenic fish has apparently taken place in some countries such as Chile, China, Cuba and New Zealand, and the legal consumption of transgenic fish in the US will likely occur soon. However, in locations such as Europe and Japan, conservative approaches to the development of transgenic fish will prevail politically for many more years. Because of these concerns, transgenic fish will likely be utilized commercially to a greater extent in developing countries than developed countries in the short term (Bartley and Hallerman 1995).

In North America marketing of transgenic salmon may be close, following submission of an application by A/F Protein, Aqua Bounty Farms, Waltham, MA, to the US Food and Drug Administration to gain approval sell GH transgenic salmon which contain genetically modified growth hormone genes (Niiler 2000). FDA approval for consumption of these fish is expected in 2004. Aqua Bounty has potential licensees for their salmon in Scotland, New Zealand, and the United States, and they expect to add others in Canada, Chile and Europe. Stocks of these same growth enhanced salmon were also eliminated in New Zealand although near commercialization because of environmental concerns, public opinion and business reasons.

Apparently, transgenic carps have been commercialized in China, and transgenic Nile tilapia in Cuba, although this is disputed.

The first commercial transgenic fish in the US may be zebrafish. Transgenic fish expressing various fluorescent pigments are scheduled for marketing in the ornamental fish industry in early 2004.

Problems encountered

Transgenic fish research and application of transgenic fish has not progressed as rapidly as many may have envisioned when research on transgenic fish began about 19 years ago. These expectations were likely unrealistic from the start. Lack of funding has been one problem hindering progress as it has for many other research areas and in particular for aquaculture and aquaculture genetics research. Although it is quite feasible to accomplish gene transfer in commercial species of fish, research facilities adequate to produce and confine transgenic fish are relatively few. Mosacism in the parent generation of transgenic fish lengthens and slows research projects. Many commercial species have relatively long generation intervals compared to species that serve as laboratory fish models and other experimental models such as mice and drosophila. Not surprisingly, lack of control of where in the genome transgenes are inserted and other possible genetic factors such genetic background and epistasis have lead to variable responses in individual transgenic families or lines. This dictates combining transgenesis with traditional techniques such as selection to identify the high performance transgenic lines and optimize their gene expression and phenotype. This makes gene transfer a medium to long term breeding program rather than a short term one. Most research institutions and scientists do not have the facilities, commitment or patience for these relatively long experiments just as is the case for traditional selective breeding and this is further aggravated by the potentially controversial nature of the research.

Some transgenic technologies have not been pursued for fish because of a lack of embryonic stem cell lines for fish. This has hindered efforts to conduct gene knockout research and homologous recombination. However, transplantation of primordial germ cells is now possible (Takeuchi 2003) opening the door for new gene knockout technology.

Early transgenic fish was also hindered by a lack of fish promoters and much of the early research was conducted with viral promoters. If commercialization is the objective, much of that early research needs to be repeated examining expression with fish promoters which will likely receive much better public perception and marketability.

Despite these obstacles, in actuality, transgenic fish research has made very good progress and results have, in some cases, been dramatically successful. As discussed later, the larger problems are overcoming environmental risk issues, food safety and public perception, which are in many ways more problematic than the obstacles encountered in the research.

Research activities

Currently, the largest research global activity that is relevant to current and future transgenic research is genomics. Large-scale research in underway regarding gene expression and regulation, gene isolation and sequencing and gene mapping.

A much smaller research effort currently exists specifically for gene transfer and transgenic application in fish, and research monies for this area are much smaller than for genomics. However, there are major ongoing projects examining enhancement of growth, disease resistance, altering temperature tolerance, sterilization or limiting of reproduction and alteration of color in fish. Additionally, ongoing research exists for modeling of environmental risk, measuring fitness and actual environmental risk and determination of food safety.

Inducible and tissue specific expression is likely needed for better transgene expression and performance in the future. Progress in being made as several tissue specific promoters have been

developed from zebrafish (Gong et al. 2002) including epidermis specific keratin 8, fast muscle specific myosin light polypeptide 2, and pancreatic exocrine cell specific elastase B. For some applications, inducible promoters may be desirable to allow induction of transgene expression at specific developmental of life stages. The inducible HSP70 gene that encodes an enzyme playing an essential role in protein metabolism has been isolated and characterized from *O. mossambicus,* and dramatically increased its rate of mRNA transcription when fish were exposed to a transient heat shock (Molina et al. 2000).

Future perspectives

Currently, there is not a great amount of research on transgenic fish throughout the world. However, there is a tremendous worldwide investment in genomics research. Funding agencies are comfortable with this area of research partially because it is not perceived as controversial. Much is being learned about genetic mechanisms and gene expression regarding the physiology, response to stressors and response to environmental variables by fish. However, the two major choices for application of this basic knowledge are marker-assisted selection and gene transfer. Although, there are other potential methods to use a portion of this information in practical ways in aquaculture, to capture maximum benefit from genomics the problems facing commercial application of transgenic fish must be overcome.

One of the greatest future potential benefits of gene transfer in fish will be enhancement of disease resistance in fish. In general, diseases are the greatest problem facing aquaculture and damaging its profitability. Additionally, this should be an animal welfare issue. Transgenic fish with enhanced disease resistance would increase profitability, production, efficiency and the welfare of the cultured fish. Preliminary research indicates great promise for success of this approach for enhancing disease resistance. Genetic gain is also possible through traditional selective breeding, but it appears that the rate of genetic improvement and the consistency of genetic improvement may be greater with the transgenic approach (Dunham et al. 2002). Selective breeding may also have the drawback that the disease organisms may well respond to selective forces as well, negating some of the selection response in the fish.

Growth rates of fish have been dramatically altered by transfer of growth hormone gene constructs. Once application of these fish are allowed, major impacts on aquaculture production can be expected. It is also apparent that transgenesis alters body composition. Data to date indicates that this has the potential for future positive impact.

Initial experiments indicate the possibility of controlling reproduction via transgenesis. Future success in this area would potentially have one of the greatest impacts from recombinant DNA technology. This approach could solve not only many transgenic issues, but many biodiversity and genetic biodiversity issues as it would allow environmentally safe application of transgenic fish, interspecific hybrids, domestic fish in general, exotic species and utilization of wild conspecifics outside their native watershed for recreational applications without genetic consequences.

Apparently, one of first applications of transgenic fish will be alteration of color of ornamental and aquarium fish with fluorescent pigment genes. If consumers have a demand for these other color altered transgenic fish, this could evolve into a major application of transgenesis with large economic impact. This may also result in additional environmental risk issues and confinement issues. Many but not all ornamentals can not survive in the natural environment. Large aquaculture facilities can be monitored to ensure adequate confinement is utilized. However, it will be impossible to monitor thousands or millions of households regarding confinement, therefore new issues and perspectives might need to be addressed.

A topic that is generally avoided is the application of transgenic fish in recreational fisheries as this would sometimes involve release of transgenic fish in unconfined areas, but also may involve release of fish in confined, urban environments. The growth rate and aggressiveness that has been demonstrated for some transgenic fish would be desirable in some sport fish applications. Public

opinion will vary in regards to this application. Some fishermen feel that they are purists, and would never want to fish for a genetically modified fish of any type. Other fishermen have expressed interest in the possibility of genetically modified trophy fish. Other fisherman would not care about either of these previous scenarios/issues, but have the objective of having a successful fishing trip, in other words having an acceptable catch rate per unit effort. Application of transgenic fish for aquaculture and ornamental fish purposes will likely occur much earlier than any recreational fisheries application.

The future success and application of transgenic fish will be dictated by successful demonstration of a lack or potential lack of environmental risk, food safety, appropriate government regulation and labeling , public education and development of genetic sterilization for transgenic fish. Initial surveys indicate that in many countries the general population does not understand biology and food production. This could exasperate marketing of transgenic fish products that are proven to be safe, although many transgenic food products partially derived from transgenic plants are currently being marketed in the US without great public outcry. When appropriate, well executed public education may be necessary to gain broad consumer acceptance of transgenic fish from an environmental standpoint, a food safety standpoint and perhaps in relationship to how "organic" a transgenic fish may be.

Data to date indicates that transgenic fish have inferior fitness traits needed for successful establishment if accidentally introduced to the natural environment. Likely, the most desirable transgenic genotypes for aquaculture will be strongly selected against in natural settings. The greater the phenotypic change for target traits such as increased growth rate, the greater the pleiotropic effects on other traits including fitness traits, such as predator avoidance and swimming ability and the decreased probability of genetic impact on wild populations. In reality, transgenic fish may be a more acceptable aquaculture genotype than traditional domestic fish, as high performance transgenics may actually be eliminated in the natural environment more rapidly than other types of domestic fish reducing impact on native populations.

However, it will be difficult to prove these hypotheses without an actual escape event. Even with strong data indicating the likelihood of low or negligible environmental risk, many governments will be reluctant to allow commercialization of transgenic fish because of public perception and pressure from the media and environmental groups. The key to this issue is development of genetic sterilization. Polyploidy has been proposed as one avenue to accomplish this, however, this approach has several drawbacks. 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. Transgenic sterilization has the potential to render transgenic fish sterile without the drawbacks of polypoidy. 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 contructs and appropriate physical confinement will reduce risk to such negligible levels that the benefits will be much greater than the risks.

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