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Comparison of Traditional Breeding and Transgenesis in Farmed Fish with Implications for Growth Enhancement and Fitness

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Improvements in the performance of fish species used in aquaculture are being accomplished using a variety of approaches, including both traditional and molecular genetic methodologies. Historical gains in productivity have been achieved by domestication, selection, interspecific and interstrain crossbreeding, polyploidy, and synthesis of monosex populations. More recently, transgenesis has been explored as a technique to enhance growth rate and other performance characteristics.

Domestication of species, without directed selection, can yield improvement in production characteristics. Domesticated strains of farmed fish usually grow faster than wild strains, and this effect can be achieved fairly rapidly: for example in channel catfish, *Ictalurus punctatus*, domestication can improve the growth rate by approximately 2–6% per generation. In contrast, directed selection (mass selection) for body weight in fish has resulted in an up to 55% increase in body weight after four to ten generations of selection. In channel catfish, correlated responses to selection include higher dressing percentage, but a decreased ability to tolerate low concentrations of dissolved oxygen.

Intraspecific crossbreeding can increase growth in channel catfish, common carp and salmonids, but crossbreeding does not always result in heterosis. Interspecific hybridization seldom results in overdominant performance in fish. However, one catfish hybrid, channel catfish female $\times$ blue catfish (*I. furcatus*) male, exhibits improved performance for several traits including growth, disease resistance, survival, tolerance of low dissolved oxygen, angling vulnerability, seinability, dressing and fillet %.

Ploidy manipulation and sex-control technologies have also played an important role in enhancing production performance. Induction of triploidy does not improve performance in catfish hybrids, but in salmonids triploidy can enhance flesh quality by preventing sexual maturation, although growth rate is somewhat reduced

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relative to diploids. Monosex male populations can increase growth rate in some strains of channel catfish, and monosex female populations of salmon have a reduced incidence of precocious maturation and thus overall improved flesh quality.

In comparison with traditional selective breeding, transgenesis in channel catfish can increase the growth rate by 30–40% by the introduction of salmonid growth hormone (GH) genes. For several species of salmonids, insertion of GH transgenes can result in dramatic weight increases of up to 11-fold after 1 year of growth. A variety of effects on commercially important characteristics other than growth are also observed in GH transgenic fish. Feed conversion efficiency is enhanced in transgenic catfish, common carp (*Cyprinus carpio*) and salmonids, an effect also observed in catfish improved for growth by traditional breeding approaches. Transgenic catfish and carp have increased protein levels and decreased fat, however, alterations in ratios among amino acids and among fatty acids in the flesh are slight or non-existent. Transgenic catfish demonstrate improved flavour and sensory scores, and GH transgenic common carp display improvements in dressing %.

Due to the potential for farmed transgenic fish to escape into natural ecosystems and breed with wild conspecifics, research has also been conducted into examining the fitness (morphological, physiological and behavioural characteristics) of transgenic animals relative to wild-type. As has been observed in other transgenic systems, body shape and physiological performance is altered in transgenic fish. In common carp, transgenic animals have larger heads, and deeper and thicker bodies. In GH transgenic salmonids, morphological disruptions analogous to acromegaly can be observed in the cranium of individuals with extraordinary growth rates. While reproductive traits are not affected in transgenic channel catfish and common carp, GH transgenic common carp display enhanced disease resistance and tolerance of low oxygen levels which could affect their ability to survive in natural systems. Foraging ability of transgenic and control catfish is similar, and under conditions of competition and natural food source, as would be the case in nature, growth is not different between transgenic and control catfish. Predator avoidance was also slightly impaired for GH transgenic catfish compared with control individuals. Swimming ability is reduced in transgenic salmon, which has implications for foraging ability and predator avoidance, as well as ability to complete arduous river migrations for spawning. Although transgenic fish may be released to nature by accident, it appears that ecological effects of transgenic fish developed and evaluated to date will be unlikely because of these examples of reduced fitness. However, each new variety of transgenic fish should be evaluated for potential environmental risk prior to utilization in aquaculture.

Great potential exists to improve production characteristics by transgenic and other molecular genetic approaches. However, future genetic improvements will continue to be achieved from traditional approaches, and, by utilizing a combination of both approaches simultaneously, maximum genetic gain should be accomplished.

Introduction

With the global escalation of human populations, world requirement and demand for high-quality protein are rising dramatically and an increasing proportion is being derived from aquatic sources. Currently, the quantity of animal protein harvested from global aquatic sources via the capture of

natural fish populations is maximal: many major fish stocks are showing precipitous declines in harvest yield due to overfishing, and further increases in ocean productivity are not anticipated under the current global climate regime. As a consequence, future demands for aquatic protein must be met through the development of aquaculture-based production systems. In 1993, approximately 16 million tonnes of aquacultured animal protein was produced, representing some 13% of the total aquatic animal protein harvested or produced (Tacon, 1996). The production of aquacultured animal protein has been increasing at a rate of over 10% annually since the mid-1980s, compared with the more modest growth of terrestrial meat production which ranges from 0.7% (beef) to 5.2% (poultry). Similar trends are also observed on a smaller scale for the aquaculture production of various finfish species (e.g. catfish, salmonids) in North America, however, it should be noted that the most rapid growth of aquacultured finfish production is occurring in developing (11.5% annually) rather than developed (4.0% annually) countries (Tacon, 1996).

With increased demand for aquacultured foods has come a need for developing more efficient production systems. Major improvements have been recently achieved through enhanced husbandry procedures, improved nutrition, enhanced disease diagnostics and therapies, and the application of genetics to improve production traits. More recently, biotechnology has begun to play a role with the isolation of piscine genes influencing a variety of physiological processes (Donaldson and Devlin, 1996) and marker DNA sequences linked to particular production characteristics (e.g. sex differentiation; Devlin *et al.*, 1994a). Gene transfer methodologies have been explored in fish since the mid-1980s, both in model systems to study basic biological processes and in fish species that are cultured for food production (see reviews by Fletcher and Davies, 1991; Hackett, 1993; Gong and Hew, 1995; Devlin, 1996). Whereas the goals of transgenic fish research do not differ from those of traditional genetic selection (i.e. improvement of production efficiency, and enhancement of product quality or character), the scope for altering these traits has been enhanced dramatically by transgenesis due to the rapidity with which responses can be achieved and by the use of genes for useful biological processes not found in the host fish species (e.g. unique disease resistance genes found only in other phyla).

Several major areas of investigation are now under way in fish genetic research, based on the needs of aquaculture production systems. We briefly review some of these approaches using examples derived from work with channel catfish, common carp and salmonids.

Domestication

When wild fish are moved from the natural environment to the aquaculture environment, a new set of selective pressures are exerted on the population

which result in changes of gene frequencies and consequent performance of the population. This process, termed domestication, occurs even without directed selection by man. Domestication effects are dramatic and can be observed in fish in as little as one or two generations after removal from the natural environment. Domesticated strains of fish almost always exhibit better performance than wild strains when placed in the aquaculture environment (Dunham, 1996). Domestication results in an increased growth rate of 3–6% per generation in captivity for channel catfish, *Ictalurus punctatus* and, not surprisingly, the oldest (86 years) domesticated strain of channel catfish (Kansas strain) has the fastest growth rate of all strains of channel catfish. Utilization of domestic strains instead of wild strains, and use of established, high-performance strains, are the first steps in applying genetic principles for improved fish culture management. Domestication will continue to play a key role in the establishment and expansion of aquaculture production as new species and strains are developed.

Strain Evaluation

Large strain differences exist for several traits of cultured fish. Channel catfish strains differ in growth, disease resistance, body conformation, dressing percentage, seinability, vulnerability to angling, age of maturity, time of spawning, fecundity and egg size (Dunham and Smitherman, 1984; Smitherman and Dunham, 1985). Similarly, rainbow trout (*Oncorhynchus mykiss*) strains also vary for numerous traits such as growth, feed conversion efficiency, survival, disease resistance, time of spawning, fecundity, hatching success and angling vulnerability (Kincaid, 1981).

Selection

Genetic selection has been a powerful tool for the improvement of production characteristics of agricultural species (crops, terrestrial animals and, more recently, aquatic species). Selection has allowed the development of several lines of fast-growing channel catfish, with the fastest growing select lines being derived from the fastest growing strains (Smitherman and Dunham, 1985) and growing twice as fast as average (Burch, 1986). For example, body weight of channel catfish has been improved by 12–20% in only one or two generations of mass selection (Bondari, 1983; Dunham and Smitherman, 1983a; Smitherman and Dunham, 1985). Similarly, after three generations of selection, the growth rate was improved by 20–30% when grown in ponds (Rezk, 1993), and four generations of selection in the Kansas strain resulted in a 55% improvement in growth rate (Padi, 1995).

In rainbow trout, six generations of selection increased body weight by 30% (Kincaid, 1983), whereas ten generations of selection of coho salmon

resulted in an increased growth rate of 50% (Hershberger *et al.*, 1990). A single generation of selection of Atlantic salmon increased body weight by 7% (Gjedrem, 1979). Crandall and Gall (1993) have shown that heritabilities for body weight in rainbow trout can be high, ranging from 0.89 in the first generation to 0.27 in the second.

Different strains of fish may possess varying amounts of additive genetic variation which can affect responses to selection. Smisek (1979) estimated heritabilities for body weight of 0.15–0.49 in a Czechoslovakian strain of common carp, and Vietnamese common carp have demonstrated significant heritability (0.3) for growth rate (Tran and Nguyen, 1993). Responses can also differ depending on the direction of selection: the body weight of common carp in Israel was not improved over five generations, but could be decreased in the same strain selected for small body size (Moav and Wohlfarth, 1974a).

Body conformation of fish can be dramatically altered by selection. Rainbow trout selected for high growth rate have a higher weight to length ratio (condition factor) relative to wild strains. For common carp, heritability for body depth was high (0.40–0.80) and, as seen in rainbow trout, deep-bodied lines have been developed (Ankorion, 1966). Selection programmes for carcass quality and quantity have also been initiated for salmonids and catfish (Dunham, 1996). Heritabilities for fat percentage in catfish and trout are about 0.50, indicating that selection should work to decrease fat content. However, heritability estimates for dressout percentage are near zero for these two species, indicating that selection for this trait would probably be unsuccessful.

Intraspecific Crossbreeding

Interstrain crossbreeding plays an important role in many animal breeding programmes, including those for fish. Heterotic growth in excess of both parent strains occurred in 55% and 22% of channel catfish and rainbow trout crossbreeds evaluated, respectively (Dunham and Smitherman, 1983a; Dunham, 1996), whereas chum salmon crossbreeds have not shown increased growth rates (Dunham, 1996). Crossbred channel catfish can grow 10–30% faster than the largest parental strain, and some common carp crossbreeds also expressed heterosis in a low percentage of the crosses examined (Moav *et al.*, 1964; Moav and Wohlfarth, 1974a; Nagy *et al.*, 1984). When crossbred strains do display heterosis, they can play an important role in commercial strain development: some heterotic crossbred strains exhibiting heterosis are the basis for carp industries in both Israel and Vietnam.

Domestication is also an important factor in crossbreeding. Domestic $\times$ domestic channel catfish crosses were more likely to exhibit heterotic rates of growth than domestic $\times$ wild crosses (Dunham and Smitherman, 1983b).

Domestic crosses of rainbow trout were also more likely to express heterosis than domestic × wild crosses (Gall, 1969; Gall and Gross, 1978; Kincaid, 1981; Ayles and Baker, 1983).

Interspecific Hybridization

Many fish species possess abilities to produce viable offspring in crosses with other species within or close to their own genera and, while most hybrids do not perform well, performance traits can be affected favourably in some cases. For example, the channel catfish female × blue catfish male hybrid is the only one of 28 catfish hybrids evaluated that exhibited overdominance for economic traits with potential applications for aquaculture (Smitherman and Dunham, 1985). This channel–blue hybrid has increased growth, growth uniformity, disease resistance, tolerance of low oxygen, dressing percentage and harvestability. However, mating blocks between the two species have thus far prevented their commercial utilization. Characteristics of reciprocal catfish interspecific hybrids can also differ. Paternal predominance (where the hybrid possesses traits more like the male parent than its reciprocal) was observed in channel–blue hybrids (Dunham *et al*., 1982).

Several salmonid hybrids have also been evaluated. As expected, salmonid hybrids were more viable when made within genera than between genera (Chevassus, 1979), but salmonid hybrids have not been found to express heterosis for growth rate. Some diploid salmonid hybrids are potentially valuable because of disease resistance inherited from the parent species that is usually not cultured, but these hybrids unfortunately have low viability. The synthesis of triploids containing one genome from the paternal species and two from the maternal species can increase the hatchability of these potentially important hybrids (Parsons *et al*., 1986).

Correlated Responses

In many cases, selection for one trait will affect other traits in either positive or negative ways because the genetic and physiological processes affecting them are linked. For example, in rainbow trout, genetic correlations of spawning date and spawning size, egg size and egg volume have been observed (Su *et al*., 1996). In channel catfish, correlated responses to selection have generally been positive: fecundity, fry survival and disease resistance all correlated positively to selection for increased body weight in channel catfish after one generation (Dunham and Smitherman, 1983a; Smitherman and Dunham, 1985), whereas dressout percentage, visceral percentage, head percentage, seinability, spawning date, spawning rate, hatchability of eggs or survival of sac fry were not affected (Dunham, 1981). After three and four generations of selection for increased body weight,

increased dressout percentage, decreased tolerance of low oxygen, but no change in body composition and seinability were observed (Rezk, 1993; Padi, 1995). Progeny from select brood fish also had greater feed consumption and feeding vigour, more efficient feed conversion, and greater disease resistance than the random controls (Dunham, 1981; Al-Ahmad, 1983). Feed consumption had a greater effect on body weight than feed conversion (Al-Ahmad, 1983).

A positive genetic correlation between body weights at different ages in channel catfish is evident from selection experiments. Select progeny grew faster than their control population during fingerling production in the first season in all strains examined (Dunham and Smitherman, 1983a). Two of the three select groups grew more rapidly during winter, and all select lines grew slightly faster than controls during the second season of growth.

Polyploidy

Fish provide a remarkable system for ploidy manipulation which can yield very useful effects on reproductive characteristics for commercial aquaculture. In many fish species, triploid females are unable to produce viable gametes in large numbers and are therefore functionally sterile, and other traits also can be affected. Triploidy can be induced by either a temperature or pressure shock soon after fertilization to block extrusion of the second polar body. Tetraploid individuals can be produced by disrupting the first mitotic cleavage using similar simple physical treatments, and these animals produce diploid sperm and thus triploid offspring in crosses to regular diploid animals.

Channel catfish triploids become larger than diploids at about 9 months of age (at a weight of approximately 90 g) when grown in tanks (Wolters *et al.*, 1982), slightly later than the time that sexual dimorphism in body weight is first detected in channel catfish. However, genotype–environment interactions occur for growth rate in triploid channel catfish: when grown in earthen ponds, triploids are smaller than diploids after 18 months at a weight of approximately 454 g (Dunham and Smitherman, 1987).

Triploid channel catfish convert feed more efficiently than diploids in a tank environment (Wolters *et al.*, 1982), and have 6% more carcass yield than diploids when 3 years of age (Chrisman *et al.*, 1983) due to the lack of gonadal development in triploids. Other traits of triploids are affected, including a darkening of the natural pigmentation. Combining triploidy and hybridization in catfish has not provided strains that grow as rapidly as diploids in commercial settings, and they have no dressout advantage and have decreased tolerance of low dissolved oxygen relative to diploids. Thus, little or no advantage exists for using polyploid catfish.

The flesh quality of triploid rainbow trout females is improved relative to diploid females because postmaturational changes are prevented (Bye

and Lincoln, 1986). Sex-reversal and breeding for production of monosex female and all-female triploid populations of trout is being evaluated with the goal of producing rainbow trout with both superior growth rate and flesh quality. Coho salmon triploids have reduced growth rates and survival relative to diploids (Withler *et al.*, 1995), but growth and survival of triploid Atlantic salmon was the same as diploids in both communal and separate evaluation (Dunham, 1996). Triploid salmonid hybrids have shown similar (Quillet *et al.*, 1987) or slower (Parsons *et al.*, 1986) growth than diploid hybrids but, similar to intraspecific triploids, interspecific salmonid triploids could grow faster than controls once the maturation period was reached (Quillet *et al.*, 1987). The rainbow trout × coho salmon triploid had decreased growth, but had increased resistance to IHN virus (Dunham, 1996).

Sex Reversal and Breeding

In many commercial agricultural species, one sex possesses more favourable production characteristics than the other, and it is often necessary to cull the population early to improve production efficiency. In contrast, single-sex populations of many fish species have been developed due to their labile sex determination process which can be influenced by exogenous sex steroids. For example, treatment of mixed-sex populations of salmonids with α-methyltestosterone produces regular XY males and sex-reversed XX males, the latter can be simply identified using sex-specific DNA markers and then used in crosses with regular XX females to produce all-female production populations (Devlin *et al.*, 1994a). All-female populations of salmonids are desirable because males display early maturity at a small size, and poorer flesh quality. A combination of sex-reversal and breeding to produce all-female XX populations is the basis for more than half the rainbow trout industry in the UK (Bye and Lincoln, 1986), as well as the chinook salmon industry in Canada. Monosex chinook salmon and coho–chinook hybrid salmon have also been produced (Hunter *et al.*, 1983).

All-male progeny would be beneficial for catfish culture since males grow 10–30% faster than females, depending upon strain (Benchakan, 1979; Dunham and Smitherman, 1984, 1987; Smitherman and Dunham, 1985). Sex reversal and breeding has allowed production of YY channel catfish males that can be mated to normal XX females to produce all-male XY progeny. Channel catfish were feminized with β-oestradiol (Goudie *et al.*, 1983), and XY phenotypic females (identified through progeny testing) were found to be fertile (Goudie *et al.*, 1985). The sex ratio of progeny from matings of XY female and XY male channel catfish was 2.8 males:1 female, indicating that most, if not all, of the YY individuals are viable. YY males are also viable in salmon and trout, Nile tilapia, goldfish and channel catfish (Donaldson and Hunter, 1982).

Genotype–Environment Interactions

Genotype–environment interactions are prevalent in aquaculture. Strains and select lines of channel catfish performed similarly in aquaria, cages and ponds (Smitherman and Dunham, 1985), and strains selected for increased body weight at one stocking density in ponds also grew faster than their control populations at other stocking densities (Brummett, 1986).

Genotype–environment interactions are large and significant when comparing growth of different species, intraspecific crossbreeds, interspecific hybrids or polyploids of catfish (Dunham, 1996). The best genotype for ponds, the channel catfish female × blue catfish male, has mediocre growth in aquaria, tanks and cages. The behaviours, nervousness and aggressiveness, are the factors causing genotype–environment interactions for the channel–blue hybrid and triploid channel catfish, respectively. Additionally, genotype–environment interactions can be related to low oxygen levels when comparing channel–blue hybrids and their parents.

Genetic Engineering

Recombinant DNA technology and genetic engineering are biotechnologies that began to be applied to aquacultural species in the 1980s, and are now complementing traditional breeding programmes for improvement of culture traits (Houdebine and Chourrout, 1991). A variety of techniques have been explored to introduce new DNA sequences into fish, including microinjection, electroporation, retroviruses, and liposome-mediated and biolistic transfer methods (Lin *et al*., 1994; Gong and Hew, 1995). Since fish embryonic stem cells are still in the early stages of development (Wakamatsu *et al*., 1994; Sun *et al*., 1995), techniques have focused on direct transfer of DNA into gametes or fertilized eggs to produce transgenic embryos.

A number of genes have been transferred into fish. Early reports of transgenesis in fish were complicated by a lack of distinction between extrachromosomal persistence of DNA early after injection, and DNA integration into the host genome. Subsequent analyses in several fish systems have demonstrated that foreign DNA can be inserted into the genome, and Southern blot analysis has indicated that the DNA can be found at one or more loci (Dunham *et al*., 1987, 1992; Guyomard, 1989a,b; Penman *et al*., 1991). Copy numbers can range from one to several thousand at a single locus, and 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.

Probably due to the cytoplasmic nature of DNA injection, virtually all founder transgenic fish are mosaic and the integrated DNA is found only in a subset of developmental cell lineages. Mosaicism has been demonstrated

in somatic tissues based on molecular tests, and also can be inferred for the germline based on the observed frequencies of transgene transmission to F1 progeny being less than at Mendelian ratios. 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 (see Devlin, 1996). 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.

A variety of promoters have been shown to be active in fish cells, and most investigations have used promoters derived from non-piscine vertebrates and their viruses. Among others, reporter gene activity has been detected in fish or fish tissue culture cells from the Rous sarcoma virus long terminal repeat (RSV-LTR), simian virus SV40, cytomegaloviruses CMV-tk and CMV-IE, MMTV, polyoma viral promoters, human and mouse metallothionein, and human heat shock 70 promoters (Hackett, 1993).

Piscine genes and their promoters also have been utilized in fish transgenesis and in cell transfection studies. Promoters from flounder antifreeze, carp β-actin, and salmonid metallothionein-B and histone H3 have been found to be active (Liu *et al*., 1990; Gong *et al*., 1991; Chan and Devlin, 1993). In general, it appears that many eukaryotic promoters are able to function in fish cells, although if derived from non-homologous sources, the level of expression may be somewhat reduced.

Expression of uninterrupted coding regions from prokaryotic and eukaryotic sources has been successful in fish cells (e.g. Du *et al*., 1992). However, it should be noted that expression in fish cells of gene constructs containing mammalian introns might be inefficient due to difficulties in RNA processing to yield functional mRNA (Bearzotti *et al*., 1992; Bétancourt *et al*., 1993). At present it is not possible to generalize about the activities of various gene promoters and gene constructs in different fish species. Such information needs to be empirically derived for the fish system of interest.

Biological Effects of Transgenesis

Positive biological effects have been exhibited by transgenic fish. Due to the lack of available piscine gene sequences, transgenic fish research in the mid-1980s utilized existing mammalian GH gene constructs, and evidence for growth enhancement was reported for some fish species examined (Zhu *et al*., 1986; Enikolopov *et al*., 1989; Gross *et al*., 1992; Lu *et al*., 1992; Zhu, 1992; Wu *et al*., 1994). Peculiarly, mammalian gene constructs (e.g. mMT/rGH) failed to have any effect on growth of salmonids (Guyomard *et al*., 1989a,b; Penman *et al*., 1991), despite a comprehensive literature showing that salmonids are very responsive to growth stimulation by exogenously administered GH protein (McLean and Donaldson, 1993). This

lack of response by salmonids to mammalian GH gene constructs may be due to difficulties associated with RNA processing as mentioned above, or due to the strains of trout utilized (see below).

Some investigators have developed gene constructs containing fish GH sequences driven by non-piscine promoters, and have observed growth enhancement in transgenic carp, catfish (Fig. 15.1), zebrafish and tilapia (Zhang *et al*., 1990; Dunham *et al*., 1992; Chen *et al*., 1993; Zhao *et al*., 1993; Martinez *et al*., 1996). Growth stimulatory effects observed with the above constructs have ranged from no effect to approximately twofold increases in weight relative to controls, and provided the first convincing data demonstrating that growth enhancement in fish can be achieved by transgenesis.

More recently, GH gene constructs have been developed that are comprised entirely of piscine gene sequences. This was achieved 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. When introduced into salmonids, such gene constructs elevate circulating GH levels by 40-fold in some cases (Devlin *et al*., 1994b; Devlin, 1996), and result in approximately a five- to 11-fold increase in weight (Fig. 15.1) after 1 year of growth (Du *et al*., 1992; Devlin *et al*., 1994b, 1995a). These GH gene constructs also induce precocious development of smoltification, a physiological transformation necessary for marine survival of salmonids.

Pleiotropic Effects

When a gene is inserted with the objective of improving a specific trait, that gene may affect more than one phenotypic character. Since pleiotropic effects could be positive or negative, it is important to evaluate commercially important traits in transgenic fish in addition to the trait intended for alteration.

The insertion of a rainbow trout GH transgene for growth enhancement may alter the survival of common carp. The number of F2 progeny inheriting this transgene is much less than expected, and differential mortality or loss of the transgene during meiosis are likely explanations. From fingerling size onwards, 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 and dropsy (Chatakondi, 1995). Where examined, reproductive traits such as fecundity or precocious sexual development in transgenic common carp, have not been affected in transgenic fish.

Increased growth rate of GH transgenic fish could arise from increased food consumption, feed conversion efficiency or both. Fast-growing transgenic common carp containing rainbow trout GH gene had lower feed conversion efficiency than controls (Chatakondi *et al*., 1995) whereas other

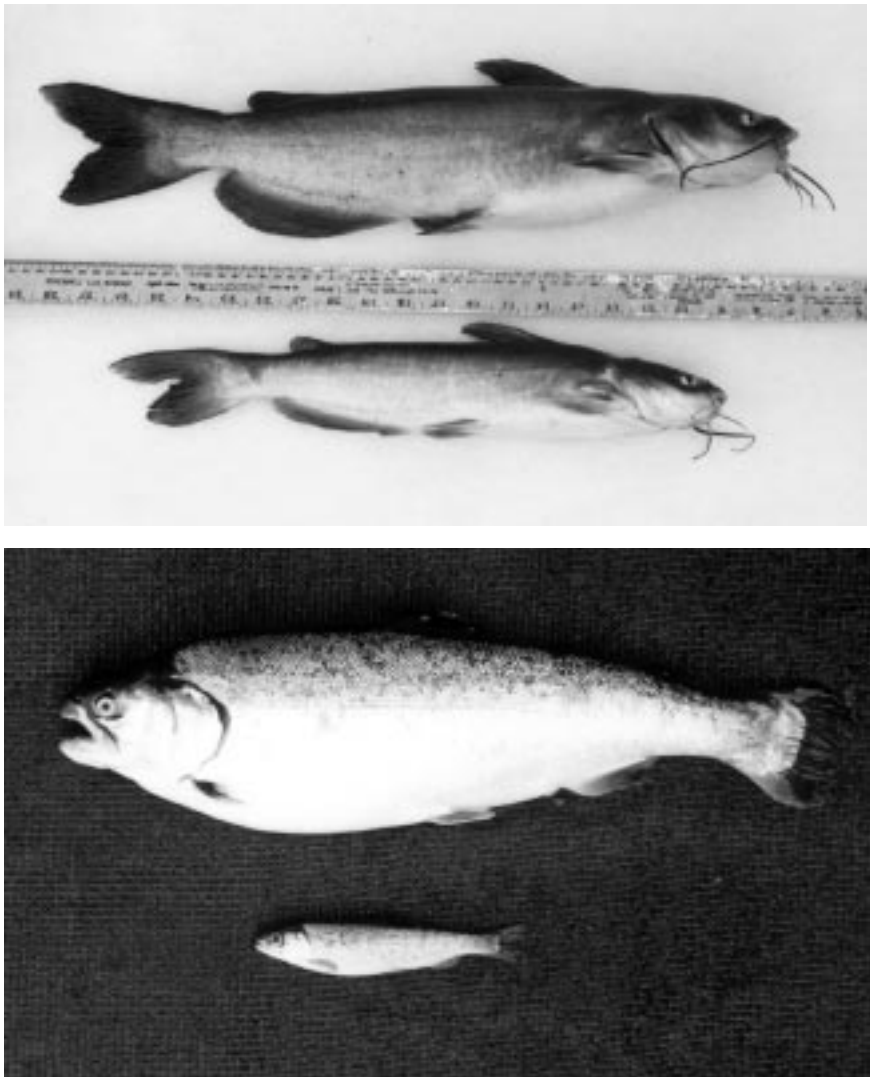

Fig. 15.1. Growth-enhanced transgenic and control catfish (top panel) and coho salmon (bottom panel). For each species, transgenic individuals are shown on top relative to control animals of the same age (approximately 1 year of age for both species). Fish sizes: transgenic catfish 900 g, control catfish 350 g; transgenic salmon 730 g, control salmon 15 g. The gene construct used in catfish is RSV LTR-rtGH1 (Dunham *et al*., 1992), and for salmon is OnMTGH1 (Devlin *et al*., 1994b).

families had increased, decreased or no change in food consumption, and had improved feed conversion. Salmonids injected with somatotropins also display improved feed conversion (Devlin *et al*., 1994c), and this effect is also anticipated in GH transgenic salmonids.

It is well known that GH has important physiological effects on energy absorption and utilization in vertebrates. Thus it is anticipated that GH transgenic fish may have altered body compositions relative to controls. Indeed, transgenic common carp containing a rainbow trout GH had more protein, less fat and less moisture than non-transgenic full-siblings (about a 10% change). GH promotes the synthesis of protein over fat, and the elevated levels of GH in transgenic fish thus increase the protein/lipid ratio, with fat level decreased by as much as 50%. The increased relative level of protein in transgenic common carp muscle also results in increased levels of amino acids, but the ratios among different amino acids and different fatty acids are virtually identical in control and transgenic common carp (Chatakondi *et al.*, 1995).

In GH transgenic salmon, the endocrine stimulation can be elevated to pathological levels in some cases, and excessive and deleterious deposition of cartilage analogous to the mammalian acromegaly syndrome has been observed (Devlin *et al.*, 1995b). This effect can be sufficiently severe such that impaired feeding and respiration may result in reduced growth and poor viability. Consequently, animals that ultimately display the greatest growth enhancement as adults are those that have been only moderately growth stimulated (Devlin *et al.*, 1995a).

In common carp, body shape changes as a result of expression of rainbow trout GH. The transgenic individuals have relatively larger heads, deeper and wider bodies and caudal areas when compared with controls. As growth differences increase, the body shape differences also increase to a point, and then plateau. The morphological change does not affect condition factor, but does improve the dressing percentage (Chatakondi *et al.*, 1994).

Environmental Risk and Fitness of Transgenic Fish

The use of transgenic fish in aquaculture has raised concerns regarding their potential interaction with other species as well as with wild members of the same species in natural ecosystems (Kapuscinski and Hallerman, 1991). Such potential impacts would depend on the degree of phenotypic change displayed by transgenic animals, as well as their fitness relative to wild-type and the number of individuals escaping from production facilities.

Transgenic channel catfish containing salmonid GH genes grow 33% faster than normal channel catfish in aquaculture conditions with supplemental feeding, but no significant difference was observed in ponds with only natural feed, indicating equal foraging abilities between transgenic and control animals. Thus, transgenic catfish require supplemental rations to exhibit their growth potential (Chitmanat, 1996), a condition which may not exist in natural environments. Relative to transgenic individuals, non-transgenic catfish fry and fingerlings had better predator

avoidance of largemouth bass *Micropterus salmoides* and green sunfish *Lepomis cyanellus*. Spawning ability of transgenic and control channel catfish was equal, but GH transgenic Nile tilapia had decreased sperm production.

The swimming ability of some transgenic salmon is reduced compared with non-transgenic salmon (Farrell *et al*., 1997) which could lead to greater vulnerability to predators, decreased ability to capture prey, and decreased ability to successfully complete spawning migrations. The increased vulnerability to predators, impaired swimming, lack of increased growth when foraging, unchanged spawning percentage and potential decreased sperm production indicate that some transgenic fish may not compete well under natural conditions which cause major ecological or environmental damage. However, due to the difficulty in predicting potential environmental impacts from laboratory fitness estimates, it would be desirable to implement physical and biological (sterilization) containment methods to reduce potential interactions between transgenic and wild fish populations (Devlin and Donaldson, 1992).

Dramatic Growth of Transgenic Fish: Explanations and Limitations

It is interesting to speculate why growth enhancement varies greatly among different transgenic fish systems (Devlin, 1996), and why salmonids in particular have shown the greatest response to stimulation to date. Several potential explanations exist which indicate that it may be difficult to duplicate these results in other fish species.

It is possible that completely homologous gene constructs (i.e. derived only from the same species, or from piscine sources) such as those used successfully in salmonids are expressed in fish more efficiently than gene constructs derived from other vertebrates. While this probably plays a role in efficient expression, it is also very likely that the differences in growth response observed in different transgenic systems are due to the vastly different physiologies and life-history characteristics that exist among the fish species examined.

The biology of salmon and their unique physiological adaptations undoubtedly play an important role in the dramatic growth enhancement observed in transgenic animals. Growth in salmonids is normally relatively slow throughout the year, and is seasonally extremely low when water temperatures are low and food resources in nature are scarce. This low growth rate appears to be controlled at least in part by the level of circulating GH and can be dramatically stimulated with exogenous GH protein (see above) and sufficient food. Thus, the dramatic growth stimulation observed in transgenic salmonids may arise, at least in part, by the seasonal deregulation of GH expression (Devlin *et al*., 1994b, 1996) to

allow high growth rates during winter months when control animals have very slow growth rates. This winter growth may also give them a large advantage that can later be magnified (Moav and Wohlfarth, 1974b). Additionally, salmonids are anadromous, and accelerated growth in transgenics allows them to reach a size where they smolt earlier than controls. Growth in the smolt stage is naturally increased, providing transgenic individuals with a further advantage to distance themselves in growth from controls.

In contrast, other non-salmonid fish species generally possess more rapid growth, and consequently may be much more difficult to stimulate by expression of GH in transgenic organisms (Devlin, 1996). These high growth rates occur naturally in some species (i.e. tilapia), whereas in others they have been enhanced through genetic selection and many years of domestication. Strains or species that have been selected to near maximal growth rates presumably have had many of their metabolic and physiological processes optimized, and would be expected to be more difficult to stimulate by a single factor such as GH.

Domestication is also important in transgenic growth responses. In this regard, we have 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 (unpublished observation). Consistent with these observations, the dramatically growth-responsive salmon previously observed (Du *et al*., 1992; Devlin *et al*., 1994b, 1995a) were also derived from wild strains. Apparently, slow-growing wild strains can benefit much more from GH insertion than fish that already have growth enhancement from selective breeding.

By comparison, GH transgenic catfish derived from domesticated and selectively bred strains exhibit only a moderate growth enhancement (41%). However, 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), then further increases from interspecific hybridization (Jeppsen, 1995) or gene transfer, the overall growth enhancement is approximately tenfold, comparable to that observed with transgenic wild salmon. Thus the growth of wild fish apparently can be improved in one or two generations with the insertion of GH genes to the extent that would take many generations of selective breeding to achieve.

Similarly, it can be noted that dramatic growth stimulation in the mammalian system using GH transgenes has been observed in mice, but not in domestic livestock that have had many centuries of genetic selection (Pursel *et al*., 1989; Palmiter *et al*., 1992). In these domesticated and selected strains, the capacity for further growth improvement by GH may now be restricted by limitations in other physiological pathways, and other methods,

including traditional breeding methods, may yield the greatest gains. For aquacultural species that have a much shorter history of domestication and selection, future genetic improvement will likely be accomplished by utilizing a combination of both approaches simultaneously.

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