# TRANSGENIC SALMON FOR CULTURE AND CONSUMPTION

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## Abstract

Over the past 20 years we have generated stable lines of transgenic Atlantic salmon possessing either antifreeze protein (AFP) genes or a salmon growth hormone (GH) gene construct. The AFP transgene is expressed and AFP secreted into the blood of all generations to date. However antifreeze protein levels remain low and a means to improve these levels needs to be developed. Our GH transgene enhances growth rates to the point where Atlantic salmon can reach market size (4-6kg) a year earlier than can non-transgenics grown commercially in Atlantic Canada. The characteristics of the transgenic salmon are described, and the hurdles to be overcome before products derived from transgenics can take their place in the world

market are discussed (Fletcher et al 1999b).

## Introduction

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

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

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

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

This brief communication highlights our progress towards generating genetically engineered Atlantic salmon broodstocks for commercial aquaculture. Our discussion centres around personal experiences with salmon. The issues involved in the production of transgenic fish and their successful integration into the aquaculture industry involve not only science but also food safety, environmental risk assessment, animal welfare, consumer acceptance, and intellectual property protection (Fletcher et al. 1999a).

**Transgenic Salmon**

We came into the field of transgenics some eighteen years ago in response to problems faced by the aquaculture industry along the east coast of Canada. Most of these coastal waters are characterized by ice and sub-zero temperatures that are lethal to salmonids. Therefore, sea cage aquaculture of salmon is almost entirely restricted to a relatively small area in the most southerly part of the region (Hew et al. 1995). The challenge for us as scientists was to find a means of producing salmon that would avoid freezing, and thus facilitate the expansion of aquaculture and economic development throughout the entire Atlantic coastal region. The solution became evident when Palmiter et al. (1982) demonstrated the power of transgenic technology as a means of genetically improving commercially important animals.

Two potential ways in which transgenic technologies can be used to solve the problem of overwintering salmon in sea cages in Atlantic Canada are: 1) produce freeze-resistant salmon by giving them a set of antifreeze protein genes, and 2) enhance growth rates by growth hormone gene transfer so that overwintering may not be necessary.

**Antifreeze Protein Genes**

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

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

**Growth Hormone Genes**

All aquaculture ventures could benefit commercially from the development of culture species with enhanced growth rates that would reduce the time required to raise fish (or shellfish) to market size. At present, it takes approximately 16-18 months of sea pen culture to produce marketable Atlantic salmon in Atlantic Canada. If growth rates during this phase could be doubled, it may be possible to market the salmon following a single growing season and obviate the need for overwintering in sea-pens.

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

The GH transgene genomic integration frequency was similar to that observed for the AFP genes (2-3 %). All of the GH-transgenic founder fish were germ cell mosaics, and half of them failed to pass on the GH transgene to their offspring. Approximately 40% of the founder transgenic fish exhibited growth rates that were, on average, 3-6 times that of standard (control) salmon over a 30 month period. Mendelian inheritance of the GH transgene and its rapid growth phenotype was established at the $F_1$ generation and has now been demonstrated through the second, third, fourth, and fifth generations.

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

**Prospects and Expectations for the Future of Transgenics**

There is no doubt that transgenic techniques can be used to produce superior fish for domestic consumption. Such improvements could impart significant benefits to the growing world population and, at the same time, have a positive impact on the stability and preservation of the marine and terrestrial environment. However, there are a number of factors to consider and weigh before the final product can enter the marketplace, and these can be grouped under the following headings:

-Science
- BioSafety (food safety; environmental protection; animal welfare)
- Consumer acceptance

***Science***

Our lessons from salmon have taught us that:

**1.** Integration frequencies of injected transgenes into the Atlantic salmon genome will be low (2-3%). This will probably be the case when using other gene constructs with other species

**2**. The transgene can integrate into more than one chromosome, and more than one copy of the gene can integrate into a single chromosome.

**3**. The transgenes can be rearranged prior to integration, resulting in weak to no expression.

**4.** All of the founder generation fish will be somatic as well as germ cell mosaics, indicating that the transgene does not integrate into the chromosomes until as late as organogenesis.

**5.** A Mendelian inheritance pattern cannot be established until the third generation ($F_2$).

**6.** Transgenic fish homozygous for the transgene cannot be produced with certainty until the fourth generation ($F_3$).

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

***Biosafety***

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

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

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

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

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

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

In Canada the appropriate regulatory agencies for food safety and animal health are Health Canada, and the Canadian Food Inspection Agency. Environmental safety is regulated by Environment Canada and the Department of Fisheries and Oceans. In United States the appropriate agency is the Center for Veterinary Medicine within the Food and Drug Administration. In the case of transgenic salmon the transgenes and their products are considered as new animal drugs.

***Consumer acceptance***

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

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

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

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