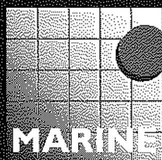
The nature and current status of Transgenic Atlantic Salmon

Reference Only

by T.F. Cross and P.T. Galvin

MARINE RESOURCE SERIES

NO. 1 1997



MARINE INSTITUTE
FORAS NA MARA

"to undertake, to co-ordinate, to promote and to assist in marine research and development and to provide such services related to marine research and development that, in the opinion of the institute, will promote economic development and create employment, and protect the marine environment"

Marine Institute Act, 1991

Marine Resource Series

The Marine Resource Series was set up by the Institute to assist in the fulfilment of its objectives under the 1991 Marine Institute Act in promoting the findings of work undertaken by, or on behalf of the Institute.

It is intended that the series will cover all aspects of marine science and technology.

Further copies of this publication, and of others in the various Marine Institute series, may be obtained from:

The Marine Institute,
80 Harcourt Street,
Dublin 2.
EIRE

Ph: + 353 1 4780333
Fax: + 353 1 4784988

Marine Institute - Marine Resource Bulletin No. 1

THE NATURE AND CURRENT STATUS OF TRANSGENIC ATLANTIC SALMON

by

T. F. Cross* & P. T. Galvin,

Aquaculture Development Centre,

Department of Zoology and Animal Ecology,

University College, Cork, Ireland

May 1996

***Phone 353-(0)21-904050, FAX 353-(0)21-270562, e.mail "t.cross@ucc.ie"**

80 Harcourt Street,
Dublin 2. Eire.
Ph: + 353 1 4780333
Fax: + 353 1 4784988

Contents

Preface 3

Current "state of the art" in salmon genetic engineering 4

What is available commercially? 7

The potential impact of transgenic salmon 8

Concerns if the strain currently being grown in Scotland were introduced to Ireland 9

Current EU Directives 10

Recommended Research 13

Acknowledgements 16

References 16

Glossary 18

Preface:

This study was commissioned by the Irish Marine Institute in response to a Ministerial request from the Department of the Marine. The definition of Genetically Modified fish (GMO) that we use throughout this report is of fish that have a gene added from the same or another species, i.e. transgenics. This is a narrow definition, in that it excludes products of **sex manipulation**[1] or **ploidy manipulation,** but is the one accepted by, for example, the European Union (Council Directive 90/220/EEC, Anon. 1990).

We were asked to address a number of questions, viz.
1. Current "state of the art" in salmon genetic engineering.
2. What is available commercially and what commercial strains would be attractive to Irish interests?
3. What is the potential impact of transgenic Atlantic salmon (*Salmo salar*) in general?
4. What particular concerns would there be if a strain currently being reared in Scotland, were to be introduced to Ireland?
5. Are current EU Directives sufficient to protect Irish interests. If not what changes should be proposed?
6. What research is required to clarify the issues?

This report was produced within twenty days of the initial request and thus we were confined largely to the literature that was available locally, though we were able to talk to some people who have various areas of expertise in the field and these people, who are listed in the acknowledgements, provided useful discussion and additional literature. One other point that should be made is that this is a very rapidly expanding field and the material described here will rapidly become dated.

1. Technical terms are indicated in **bold** and explained in the glossary.

1. Current "state of the art" in salmon genetic engineering

Before describing what is currently available in this area, it is first necessary to describe the process of transgenic induction in fish. Maclean & Penman (1990) list the following steps:-

i) Acquiring the gene
The location and mode of action of a particular gene must be known. This is the case for example, with the fish growth hormone (GH) gene. The genomic DNA or **cDNA** for a particular gene can be used. The former, being the structural gene is more likely to be functional, but may be unacceptably large due to the presence of multiple **introns,** so the trend seems to be to use cDNA (Hackett, 1993), though some recent workers use **genomic DNA** (Devlin et al. 1994).

For the gene to be active in the target fish the upstream **promoter** region must also be included, as well as a downstream **termination** sequence. In some cases enhancer sequences which lie further upstream and downstream of the promoter region are required to have full physiological function. Early transgenic work (Hackett, 1993) utilised viral promoters which were spliced to the target gene to achieve what is referred to as a gene construct. Since many of these promoters were from disease organisms their use in fish was counter indicated for human health reasons. Mammalian promoters were then used but have now largely been replaced for piscine use by fish promoters, to give what are referred to as "all-fish" **constructs**. It may be advantageous to use a mixture of gene and promoter of separate function, where the latter can be switched on at will or at a time when or in a tissue where, the target gene is not normally active. For example, a metallothionein promoter can be induced by the action of heavy metal ions, and cause a gene of alternative function to become active.

ii) Cloning the gene
The gene sequence or construct must be **cloned** into a suitable vector for introduction into a bacterial strain for subsequent multiplication. The gene is then harvested and usually cleaved from the vector using restriction enzymes to yield multiple copies of the construct.

iii) Introducing the gene into the target fish
The most common way of introducing genes into fish is by **microinjection** of newly fertilised eggs (before the sperm and egg nucleus fuse), with multiple copies of the construct. Microinjection is done via the micropyle in Atlantic salmon (*Salmo salar*) eggs, while injection through the chorion has been successful with rainbow trout (*Oncorhynchus mykiss*) eggs (Maclean and Penman, 1990). It is important to place the constructs in the close vicinity of the egg nucleus to ensure incorporation. With the large yolky eggs of salmonids it is not possible to inject into the egg nucleus. Considerable skill and training is required to achieve high levels of incorporation (*loc. cit.*).

Other methods of introducing genes like **electroporation** of eggs, the use of sperm as vectors and by particle bombardment have been discussed for use with fish, but have only been tested in a minority of cases. A method that may be utilised with fish in the near future is electroporation of sperm. It is not clear how the constructs are incorporated into the genome, i.e. whether they form parts of the chromosomes. One hundred percent incorporation is never achieved and in those individuals where incorporation does occur (usually less than 20%) **mosaicism** is the norm. This is because incorporation usually does not occur until the zygote has started to divide, so only descendants of particular cells will contain the novel gene.

iv) Assaying for transgenism
This can be done using **Southern blotting** or Polymerase Chain Reaction (**PCR**) of sacrificed fry or biopsy samples from larger fish. Different signal strength between individual fish is indicative of mosaicism.

v) Is the gene expressed?
This is normally tested for by seeking the novel protein or, if a native protein is being expressed in greater quantity, by phenotypic expression (Fletcher *et al.*, 1988).

vi) Transmission to the next generation
The presence of the novel gene in gametes is first confirmed and these fish are then used to produce progeny. Normally, non-transgenic fish are crossed with fish where **germ line transmission** has been confirmed. Equal numbers of normal and transgenic progeny would be expected if a single copy of the construct is present is each transgenic. Either the traditional quantitative approach of breeding from transgenics, preferably those with multiple copies of the trangene, or more novel techniques such as diploid gynogenesis can then be used to increase transgenic level towards 100% over a number of generations.

Penman *et al.* (1995) stated that 22 species of fish had been involved in transgenic experiments up to the time of writing and that 40 different constructs had been used. Attention has focused on a number of different functional genes (as opposed to **reporter genes** which have been used experimentally to test various promoters).

Prominent among these functional genes are the following:-

a) Growth hormone genes
These genes have been used in common carp (Zhang *et al.* 1990), rainbow trout (Penman *et al.* 1990), Pacific salmon (Devlin et al. 1994) and Atlantic salmon (Hew et al., 1995). Among salmon, dramatic growth increases have been demonstrated, with Devlin et al.(*loc. cit.*) showing that the fastest growing transgenic coho salmon of the group investigated, was 37 times heavier than the average control weight after 14 months in freshwater.

Devlin *et al.* (1995) went on to produce progeny from these transgenic coho salmon and reported fast growth but profound morphological abnormalities. There was disproportionate growth of the head and operculum cartilage disimproving appearance and leading ultimately to respiration problems. This condition is similar to **acromegaly** in humans. It was noted that the phenomenon was more serious in the F1 generation, perhaps because of mosaicism in the parents.

b) Antifreeze protein genes
These genes which were isolated from winter flounder, ocean pout and wolfish and allow these species to survive sea temperatures below -1°C by producing antifreeze proteins (Gong & Hew, 1993). These genes have been transferred to Atlantic salmon in an effort to increase tolerance to freezing conditions (Hew *et al.* 1995). The genes are active and can be passed to progeny, but are not present in sufficient numbers to increase freeze tolerance.

c) Other genes
Other genes which have been transferred in fish or are being discussed include metallothionein genes (Olssen, 1993), esterase genes, which could make farmed salmon more resistant to the organophosphates used to treat for sea lice (Maclean & Penman, 1990); disease resistance genes and non-functional segments of DNA, which could serve as a heritable internal tag for reared fish.

2. What is available commercially?

It appears from scanning the recent aquaculture magazines and from talking to experts in the area, that only one product for Atlantic salmon is currently available. This is a construct, which combines an ocean pout antifreeze promoter with a salmonid growth hormone gene. This construct is marketed by Aqua Bounty Farms, a subsidiary of AF Proteins Inc., who have offices in Massachusetts, USA and Newfoundland, Canada.

We understand that this is the construct which was injected into fertilised Atlantic salmon eggs of European farmed origin at a land based farm in Scotland (MacKenzie, 1996). We say that the eggs and milt were of European origin to distinguish them from eggs of North American origin. This point is important because there are considerable genetic differences between *Salmo salar* from Europe and North America, and intercontinental transfers are not recommended. We are not sure whether the farmed parents were of Scottish, Norwegian or mixed origin. Strains farmed in Scotland commonly come from these three sources.

The gene construct involved was brought from Canada and introduced, by microinjection, into 10,000 eggs. Percentage transgenics within the parr derived from these eggs has not been disclosed. It is unclear how many females were involved in producing this number of eggs. Given the fecundity of salmon, it could have been as few as two. Nor do we know whether rate of transgenic incorporation was equal between individuals. These points are important, because using so few females as broodstock would cause a major genetic bottleneck and much of the genetic variability present in the original population would be lost. If it were intended to use these transgenic individuals as broodstock, these efforts might be hampered by the appearance of inbreeding defects in subsequent generations. Also, any escape of such fish into the wild would lead to loss of variability in wild populations, if the transgenic fish were to breed successfully with wild salmon.

We are unclear on what it is intended to do with these fish. The Scottish farm in question, being land-based, can presumably ongrow these fish in seawater tanks, until they are sexually mature. We have heard anecdotally that it is intended to sell the resulting eggs to Chile.

The company's promotional literature states that the construct they market is, as already stated, an ocean pout antifreeze promoter spliced to a salmonid growth hormone gene. Since antifreeze proteins are produced in the liver, growth hormone will be produced there by this construct. This will be in addition to its normal site of production, the pituitary gland. Growth hormone production from the pituitary gland is inhibited by low temperature, but this will not be the case in the liver, so growth will continue throughout the winter. Thus, higher levels of growth hormone will be produced during the summer, because it is being produced in two tissues and production will continue during the winter. As far as we are aware, no other commercial transgene construct for Atlantic salmon is currently being marketed.

In principle, the construct mentioned above would be of interest to Irish salmon farmers. However, all Irish production farms currently use sea cages, from which total prevention of escapes is impossible. Use of transgenics under such circumstances is not recommended, as will be discussed below.

3. The potential impact of transgenic salmon

This subject area has been discussed by many authors (Gliddon & Goudet, 1995; Kapuscinski, 1995). Potential effects may be considered to the environment and on consumer perception.

Possible environmental effects:
Such effects will only occur when transgenic salmon escape into the wild. Most of the areas of concern in considering escapes of ordinary farmed salmon apply here. In terms of genetics, transgenic salmon are likely to be highly inbred, so would seriously compromise the levels of genetic variability in a population if they were to interbreed. Even if transgenics had reduced **Darwinian fitness**, there might still be **introgression** of genes into wild populations with unknown consequences. Second and subsequent generation hybrids might suffer from the phenomenon of **outbreeding depression** caused by the breakdown of **co-adapted gene complexes**. Large scale escapes of a single transgenic strain over an entire coastline would lead to vastly enhanced gene flow between distinct populations, thus compromising local adaptations.

All of these effects could occur if the transgenics were capable of reproduction but would be alleviated if sterile transgenics were to be produced. For this to be done successfully using current technology a two generation process is involved. All female fish are produced by sex reversing chromosomally female parents to functional males then using these to fertilise normal eggs. The resulting zygotes are then pressure or temperature shocked to yield sterile triploid all-females. Many authors have argued that all transgenic production salmon should be sterile (see for example, Penman et al. 1995). The assumption is that there is no environmental threat if such fish escape. We would contend that escaped transgenics might still have a major environmental effect particularly if they were to grow unusually fast (Hindar, 1995). The effects on a limited food supply, the success in competitive encounters and the possibility of very large fish acting as major predators are just some of the potential ecological effects which might be envisaged.

Concerns in the area of consumer perception:
Salmon relies on its natural image to attract customers. One of the major areas of contention between those who catch wild salmon and those who produce farmed salmon, has been the suggestion that "unnatural" methods are used in farming. Examples would be the use of therapeutics like malachite green, antibiotics and organophosphates in farmed production. In this context, we understand that elements within the Scottish industry have expressed concern about the use of triploids. We feel that the use of transgenics in the farming industry could elicit a major negative impact. The popular press already contains many negative articles, since the news of transgenics being held in a Scottish salmon farm became public. One of us (TFC) was contacted recently by a supplier of farmed salmon to the German market who was concerned about the adverse effects on German salmon sales of the possible use of transgenics in salmon culture. Further, we understand that many companies in the Scottish industry would not currently embrace this technology, because of fears of adverse customer reaction. The Norwegian Government, which has been extremely supportive of the growth of salmon farming, is also reported to be unlikely to grant licences for the use of transgenic salmon in aquaculture at present (Anon, 1993).

4. Concerns if the strain currently being grown in Scotland were introduced to Ireland.

All of the general concerns mentioned above would have to be addressed if the strain currently being grown in Scotland were to be introduced to Ireland. Furthermore, as we mentioned earlier, all Irish commercial salmon farms utilise cage culture and thus could not provide adequate containment. Irish authorities would need a great deal of information to even consider a request for importation.

Such details should include:
a) origin and numbers of male and female components used to found the transgenic strain;
b) details of freshwater growth and mortality of both transgenic and controls;
c) extent of mosaicism in parental transgenics;
d) extent of germ line transmission;
e) details of relative food conversion ratios of the two groups;
f) taste panel results and other nutritional parameters on the market sized fish;
g) market trial results from these transgenics;
h) environmental impact assessment results, possibly along the lines of risk assessment/management suggested by Kapuscinski (1995).

Perhaps most crucial aspect from the customer perception viewpoint is whether there is any evidence of acromegaly-like effects in transgenic parents. If so, from the experience of Devlin *et al.* (1995), it might be expected to be much more serious in the F1 generation.

5. Current EU Directives

The European Council Directive of 23 April 1990 (Anon., 1990), on the deliberate release into the environment of genetically modified organisms attempts to legislate for a diverse range of organisms modified by a range of genetic techniques. In this respect, much of the emphasis is therefore placed on the need to control GMOs which pose a potential threat to human health. While this is obviously a major concern with aquatic organisms as with any others, organisms released into the aquatic environment differ from terrestrial species in a number of aspects which are relevant to legislation of this nature. In this context, Canadian (Anon., 1994) and US (Anon., 1995) regulations are more specific.

The most important difference between aquatic and terrestrial environments is that organisms released into natural aquatic systems are not normally recoverable. Therefore, the consequences of releases of GMOs in natural ecosystems should be regarded as irreversible. This applies regardless of whether the organisms have been sterilised or not, since even sterile animals may have the propensity to severely disrupt an ecosystem, even within a single generation. Therefore, until such time as a particular strain of organism has been characterised, to the extent that there is extensive knowledge available on the potential implications of the release of that organism on relevant aquatic ecosystems, then no releases should be permitted. Since aquatic ecosystems function through complex interactions involving transfers of energy, organisms and nutrients, there will be considerable difficulty in predicting the community-level impacts of releasing transgenic fish that exhibit one or more types of phenotypic change. In its present format, the Council Directive defines a GMO on the basis of the molecular techniques by which the organism has been modified. Yet, it is the phenotypic change which is important when the organism is released into the ecosystem, regardless of the technique involved in bringing about that manipulation.

Of particular concern in the existing legislation, is the area which relates to possible disparity between the rules in operation in the different member states. In such situations, the directive states that *"the deliberate release into the environment of GMOs may create unequal conditions of competition or barriers to trade in products containing such organisms, thus affecting the functioning of the common market; whereas it is therefore necessary to approximate the laws of the Member States in this respect"*. The apparent implication from this regulation is that a compromise should be sought between the rules of the relevant Members when such a problem arises. This is not a satisfactory clause, since any compromise in the rules of a member state, will invariably mean a compromise of the ability of a member state to protect its native fish species from the possible impacts of GMOs.

A further related clause is stated as follows: *"Whereas, when a particular product containing a GMO or a combination of GMOs is placed on the market, and where such a product has been properly authorised under this directive, a Member State may not on grounds relating to matters covered by this Directive, prohibit, restrict or impede the deliberate release of the organism in that product on its territory where the conditions set out in the consent are respected: whereas a safeguard procedure should be provided in case of risk to human health or the environment"*. The implications of this clause may serve to limit the ability of individual Member States to evaluate the characteristics of particular GMOs on a "case-by-case" basis.

It is likely that over the next decade, genetic manipulation of fish species will become a more refined science, in which the "shopping list" of available gene constructs will become more extensive. It is quite conceivable that some such constructs may have attributes that could be very useful to enable the conservation of endangered wild populations; an example of such a scenario might be the development of a gene construct that would protect Norwegian salmon from *Gyrodactylus*, Irish salmon and sea trout (*Salmo trutta*) from *Lepeoptheirus salmonis*, or Baltic salmon from M74. It would be very difficult to oppose the use of such a construct where the alternative might mean the eminent collapse of the population or populations concerned. However, this should not necessitate that either the organism or the construct be freely available for use in other Member States, subject to the same conditions as for the Member State where the original problem demanded such drastic action.

With respect to the use of GMOs in aquaculture, there are three perceived situations in which these might be utilised: totally enclosed land-based systems, net-pen culture and release into open natural ecosystems. While floating net-pen culture represents an intermediate between open natural systems and the enclosed land-based systems, it is impossible to completely secure net-pen facilities and past experience has indicated that escapes invariably occur (Kapuscinski & Hallerman, 1990a & b). Therefore, regulations governing the release of GMOs to net-pens should not differ from releases to open natural systems. With respect to land-based aquaculture facilities, licences should only be granted where there is clear demonstration that the facility is secure against escape of the organism as well as unauthorised access by humans. Recent experiences with certain militant groups emphasise the need to protect the premises from sabotage, especially where such an event might lead to the release of the GMOs to the natural ecosystem. Examples of the necessary requirements include the siting of the facility away from natural flood-plains, a minimum of double screening of water input and output pipes, passage of discharged water through a gravel trap before release in natural waters, complete perimeter fencing of the facility and a prohibition of removal of live fish from the grow-out facilities (even for research purposes).

The European Council Directive (90/220/EEC-Anon., 1990)* was designed to be all encompassing for a rapidly evolving technology which had the potential to have major implications on environmental and public health related issues. However, as outlined above, there are certain difficulties with this Directive as it pertains to transgenic fish. Due to the characteristic external fertilisation of Teleost fish, they present very attractive models for experimentation with transgenic research. The likely result of this will be that there will be significant advances of transgenic technology in these species. It would therefore be appropriate to have additional or even completely separate regulations governing GMOs in aquatic systems to take these factors into consideration (Hallerman & Kapuscinski, 1990). Most importantly, regulations relating to the common market and the free movement of produce, should set conservation of natural ecosystems as the "least common denominator" for licensing of genetically modified fish. In this instance, regulations consistent with those of exotic species or the prevention of spread of diseases are more appropriate to preserve the natural ecosystem and avoid irreversible damage.

Editor' note: *The Environmental Protection Agency (EPA) have informed us that they are the Competent Authority (CA) to implement the Genetically Modified Organisms Regulations, 1994 (S.I. 345 of 1994) to implement Directives 90/219/eec on the Contained Use of GMMs and 90/220/EEC for the Deliberate Release into the environment of GMOs. The Directives were transposed into Irish law in November 1994. Commencement date for implementation was January 1, 1995. (See. Footnate - Page 15)*

For the development of research in transgenic fish, an important consideration from an environmental perspective will be to discourage research that results in large phenotypic changes in the organism. The degree of risk associated with the release of GMOs is related to the degree to which the phenotype differs from the natural strain. Until the effects of the promoter sequences in the constructs are better understood, research in this area should focus on genes which would be least likely to have a negative impact on wild populations in the event of an escape. Examples of such genes include those involved in disease resistance, tolerance of pollutants, or simply genetic tags which are non-coding. While commercial interests in aquaculture are likely to strive more towards growth related modifications, such as constructs containing a growth hormone, licensing of transgenic fish for use in any situation with even a remote possibility of an escape into the environment, even for research purposes, should be restricted initially to such "low risk" categories. It will be essential to fully characterise the effects of the promoter sequences on non-target genes and to experimentally assess the potential for introgression of the construct into the wild populations. In this way, it should be possible to gather all the necessary information to enable some degree of modelling on what the possible consequences might be of introducing constructs which result in more dramatic phenotypic effects.

6. Recommended Research

Both from an aquaculture perspective and from the context of a model organism for transgenic research, the number of transgenic fish research programmes is likely to increase substantially over the next decade. While the information relating to the production of transgenic fish is likely improve as a result, there is a definite need for a structured research plan to assimilate the necessary information to enable this research to proceed without incurring substantial risk to the environment. There is also a need to evaluate the products resulting from transgenic fish both from health and safety and from consumer satisfaction perspectives.

The first priority for research relating to transgenic fish should be to investigate the secondary effects of the promoter sequences utilised. This should be assessed by development of a construct with a promoter sequence, together with a non-coding marker sequence that would enable identification of the fish containing the construct. In this way, the potential secondary effects of each promoter could be evaluated, by measuring phenotypic changes induced by the promoter. Using **anchored PCR**, it should be possible to map insertion sites of the construct and thereby characterise potential impacts related to various insertion sites separately. This obviously needs to be done on a range of genotypes, to ensure that alternative insertion sites or genotypic compositions do not result in drastically differing impacts. In this way, a "final" strain of transgenic fish ready for field trials should be defined by the promoter and construct insertion site, thereby limiting the possible outcomes resulting from introgression.

Field trials should then be carried out using locations which have the necessary security to prevent escape of the fish from the experimental area at any stage of the life-cycle. Through simulation of an escape into such an enclosed ecosystem, it should be possible to evaluate:
a) the degree to which the transgenic fish are capable of mating with a native population;
b) the success of that mating as defined by fertilised eggs;
c) the relative fitness of juveniles of pure transgenic crosses, hybrids between native and transgenic crosses, and the pure native crosses.
It is critical to gain such information before licences should be are granted for any trials of transgenic fish which contain coding sequences.

Having defined the impacts of the promoter on its own, it will then be possible using the same methodology to evaluate the potential impacts of using the various coding sequences. While the latter could take the form of an EIA to be carried out on behalf of commercial interests in relevant strains of transgenic fish, the former will probably need to be undertaken as basic research, funded by the EU or individual governments.

Much of the risk associated with the use of transgenic fish concerns possible introgression into native populations. This can be avoided if comprehensive sterilisation methodology can be developed, i.e. 100% sterilization. The current state-of-the-art in this area does not usually provide sterilisation that is comprehensive enough to safeguard the environment. Since even a single non-sterile transgenic fish escaping into the wild and hybridising with native fish could be enough to result in severe implications, it will be necessary to develop methods that either ensure 100% sterilisation, or that enable easy detection and removal of non-sterile fish.

If adaptive transgenes are permitted to enter a wild population, the genotypes of these could, in some cases, sweep the population, eliminating other genotypes and reducing the amount of genetic variation. Even from an aquaculture perspective, it is very important to retain the genetic diversity of the wild populations that might in the future be useful for selective breeding. Therefore, until the ecological effects of each new transgene are fully quantified, all steps necessary to prevent escape and genetic introgression need to be rigidly enforced.

Thus, there is an urgent need to undertake research at a basic level, in order to obtain a background understanding of:

a) what effects the available promoters have on non-target genes,
b) what phenotypic effects result from introgression of each type of construct into the genome of natural populations;
c) what the possible ecological consequences might be on a range of different ecosystems, and
d) how the construct might affect the current range of the organism.

The latter represents a very significant threat, in which the fish species may extend its range into new ecosystems, with consequential impacts that might not be predictable on the basis of the current ecosystems occupied, e.g. salmon with an introduced antifreeze protein gene allowing northward extension of the species range (Chan *et al.*, 1993, Davies *et al.*, 1993).

Apart from the aforementioned environmental related concerns, there is considerable need for market research regarding transgenic fish. The net benefits to the producer of utilising genetically modified fish are generally cost related. However, whether resulting from greater food conversion efficiency, disease resistance, or improved growth, the changes are only beneficial if they do not affect consumer demand. It will therefore be necessary to determine how consumers view the use of transgenic research and thus the marketability of transgenic fish. If it emerges that transgenic fish can only be marketed at reduced prices, then it may be that their use is not cost effective compared to existing natural strains used in aquaculture.

Secondly, it will be necessary to investigate to what extent the introduction of transgenic fish on the market place, would affect the current perception of artificially reared species. The danger here is that all farmed strains might be grouped together by the consumer (whether transgenic or not), with the result that the demand for current farmed strains might decrease, and more importantly, demand might increase for wild salmon products. Any move in this direction would represent a reverse of trends over recent years (where the farmed strains are becoming accepted as almost equal substitutes to wild fish by the consumers). This would be very undesirable, since it would have the effect of increasing demand for wild fish. In the case of Atlantic salmon especially, this would inevitably result in an increase in price and a consequential increase in illegal fishing to meet this demand.

Thirdly, depending on the types of construct used, there may be phenotypic effects manifested in terms of the texture or taste of the fish. Consumer reaction to such changes needs to be carefully evaluated, in order to predict to what extent such changes might affect consumer demand.

The current construct being utilised for Atlantic salmon consists of an ocean pout promoter sequence together with a growth hormone gene. The extent of additional production of growth hormone needs careful examination from a health and safety perspective, as well as a market research angle. Public awareness has been heightened recently in relation to the

possible consequences of consuming products with elevated levels of hormones. Thus, there would need to be a comprehensive study to establish that the use of such transgenic fish poses no threat to public health.

There are many questions which need to be answered before it is possible to realistically assess the potential of transgenic fish. If the EU and individual governments wish to support a policy of encouraging transgenic research, then it is necessary to provide financial support for basic research as outlined above. The additional research required for any particular gene construct with a particular strain of fish, could take the form of EIA assessments, and would therefore be the responsibility of the commercial interests that stand to gain from the utilisation of the specific transgenic fish.

In conclusion, it is anticipated that there will be increasing pressure from the aquaculture industry to adopt transgenic technology. Thus, the Marine Institute should seek to have greater research priority accorded to this area at national and EC level.

Editor's Footnote

Regulation of transgenic fish under S.I. No. 345, 1994 was discussed at the Environmental Protection Agency's Advisory Committee on Genetically Modified Organisms on 6th June 1996 with the following outcome:

"The Committee agreed that transgenic fish should be regulated as a contained use under S.I. No. 345, 1994. The Committee was told that Biological containment was only 90% effective in some cases and could not be relied on to limit the environmental impact of transgenic fish escaping into the environment. It was reported that even with non-indigenous sterile females there was a significant danger that a "super-predator" would escape and thus alter the environment. The Committee was also informed on a commercial venture in Scotland where it was experimentally shown that transgenic salmon can grow 37 times faster, but on average, growth rates of 7-8 times faster were usually achieved compared to non-modified salmon. The gene construct consists of a growth hormone plus an antifreeze promoter that ensures that the hormone is produced throughout the year, thus resulting in the faster growth rate of the salmon. This construct is commercially available for use in industry. Apparently, the transgenic fish have severe physiological problems. The Committee agreed that it would be inappropriate to allow such an enterprise to start in Ireland at present, due to the potential adverse effects on the environment. However, for research and development purposes, it might be possible to consider research work on transgenic fish, providing strict containment was provided. Notifications would be treated on a case by case basis. It was pointed out that the salmon farm industry is very important in Ireland, and that the Irish Salmon Growers Association are concerned about the potential negative impacts these fish might have on the industry.

The Committee were informed that, in order to carry out this type of research in Ireland, consent must be given by the Agency and the Department of the Marine."

Acknowledgements:

We wish to thank Professor Andrew Ferguson, Queen's University Belfast, Dr. Kjetil Hindar, Norwegian Institute for Nature Research, Dr. Lindsay Laird, University of Aberdeen, Professor Jarle Mork, Trondheim Biological Station, Dr. David Penman, Stirling University and Dr. David Skibinski, University of Wales, Swansea, for useful discussion and, in many cases, for providing literature.

References

Anon. 1990. Council Directive of 23 April 1990 on the deliberate release into the environment of genetically modified organisms (90/20/EEC). *Official Journal of the European Communities*, 8.5.90, No. L 117/15.

Anon. 1993. The act relating to the production and use of genetically modified organisms (Gene Technology Act). T-950, Ministry of Environment, Norway.

Anon. 1994. Transgenic aquatic organisms: policy and guidelines for research with, or for rearing in natural aquatic ecosystems in Canada.

Anon. 1995. Performance standards for safely conducting research with genetically modified fish and shellfish. Document No. 95-01, prepared by the U.S. Department of Agriculture, Agricultural Biotechnology Research Advisory Committee Working Group on Aquatic Biotechnology and Environmental Safety.

Chan, S.L., Fletcher, G.L. & Hew, C.L. 1993. Control of antifreeze protein gene expression in winter flounder, pp. 293-306. In: *Biochemistry and Molecular Biology of Fishes*, vol. 2, Hochachka, P.W. and Mommssen, T. P. (eds.).

Davies, P.L., Ewart, K.E. & Fletcher, G.L. 1993. The diversity and distribution of fish antifreeze proteins: new insights into their origins, pp. 279-292. In: *Biochemistry and Molecular Biology of Fishes*, vol. 2, Hochachka, P.W. and Mommssen, T. P. (eds.).
Devlin, R.H., Yesaki, T.Y., Blagl, C.A., Donaldson, E.M., Swanson, P. & Chan, W.-K. 1994. Extraordinary salmon growth. *Nature*, 371, 209-210.

Devlin, R.H., Yesaki, T.Y., Donaldson, E.M. & Hew, C.-L. 1995. Transmission and phenotypic effects of an antifreeze/GH gene construct in coho salmon. *Aquaculture,* 137, 161-170.

Fletcher, G.L., Shears, M.A., King, M.J., Davies, P.L. & Hew, C.L. 1988. Evidence of antifreeze protein gene transfer in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, 45, 352-357.

Gliddon, C. & Goudet, J. 1995. Gene flow and analysis of structured populations, pp. 32-42. In *Environmental impacts of aquatic biotechnology*, OECD Documents, Paris.

Gong Z. & Hew, C.L. 1993. Promoter analysis of fish antifreeze protein genes, pp. 307-324. In: *Biochemistry and Molecular Biology of Fishes*, vol. 2, Hochachka, P.W. and Mommssen, T. P. (eds.).

Hackett, P. 1993. The molecular biology of transgenic fish, pp. 207-240. In: *Biochemistry and Molecular Biology of Fishes*, vol. 2, Hochachka, P.W. and Mommssen, T. P. (eds.).

Hallerman, E.M. & Kapuscinski, A.R. 1990. Transgenic fish and public policy: regulatory concerns. *Fisheries*, 15(1), 12-20.

Hew, C.L., Fletcher, G.L. & Davies, P.L. 1995. Transgenic salmon: tailoring the genome for food production. *Journal of Fish Biology*, 47, 1-19.

Hindar, K. 1995. Ecological and genetic effects of transgenic fish, pp. 233-244. In Council of Europe, editors. *Pan European conference on the potential long-term ecological impact of genetically modified organisms.* Council of Europe Press, Strasbourg.

Kapuscinski, A.R. 1995. Implications of introduction of transgenic fish into natural ecosystems, pp. 43-61. In: *Environmental impacts of aquatic biotechnology.* OECD Documents, Paris.

Kapuscinski, A.R. & Hallerman, E.M. 1990a. Transgenic fish and public policy: anticipating environmental impacts of transgenic fish. *Fisheries,* 15(1), 2-11.

Kapuscinski, A.R. & Hallerman, E.M. 1990b. Transgenic fish (AFS position statement). *Fisheries*, 15(4), 2-5.

Maclean, N. & Penman, D. 1990. The application of gene manipulation to aquaculture. *Aquaculture,* 85, 1-20.

MacKenzie, D. 1996. Altered salmon grow by leaps and bounds. *New Scientist.* January 6, 6.

Olsson, P.-E. 1993. Methallothionein gene expression and regulation in fish, pp. 259-278. In: *Biochemistry and Molecular Biology of Fishes*, vol. 2, Hochachka, P.W. and Mommssen, T. P. (eds.).

Penman, D.J., Woodwark, M. and McAndrew, B.J. 1995. Genetically modified fish populations, pp. 22-27. In: *Environmental impacts of Aquatic Biotechnology*, OECD Documents, Paris.

Penman, D.J., Beeching, A.J., Penn, S. & Maclean, N. 1990. Factors affecting survival and integration following microinjection of novel DNA into rainbow trout eggs. *Aquaculture*, 85, 35-50.

Zhang, P., Hayat, M., Joyce, C. Gonzalez-Villasenor, L.I., Lin, C.M., Dunham, R.A., Chen, T.T. and Powers, D.A. 1990. Gene transfer, expression and inheritance of pRSV-rainbow trout-GH cDNA in the common carp, *Cyprinus carpio* (Linnaeus). *Mol Reprod Dev* 25:3-13.

Glossary

Acromegaly: A condition in humans caused by overproduction of pituitary growth hormone where there is exaggerated growth of cartilage.
Anchored PCR: A technique which enables amplification of sequences adjoining a known segment of DNA, hence allowing the sequence composition of the flanking regions to be determined.
cDNA: DNA reverse transcribed from an RNA template
Clone: A population of identical cells, generally those containing identical recombinant DNA molecules.
Co-adapted gene complex: A combination of alleles or genotypes at different loci which result in increased fitness (analogous to combining letters of the alphabet to form a sentence) and is the basis of local adaptation.
Construct: This refers to a sequence consisting of a promoter and a target gene spliced together enzymatically. It can be inserted into another animal to produce a transgenic.
Darwinian fitness: Measured in terms of reproductive output.
Electroporation: A method of making transgenics using electrical charge to introduce the construct into the cell.
Genomic DNA: DNA extracted from cells where no selection has occurred.
Germ line transmission: Presence of a transgenic insert in the gametes which will then be transmitted to the offspring.
Introgression: The introduction of genes from one strain to another by breeding.
Introns: Non-coding region of an interrupted gene that is transcribed into RNA, but is excised during processing of the primary transcripts into mature mRNA.
Microinjection: A method for introducing new DNA by injecting it directly into the nucleus.
Mosaicism: An organism with cells of different genetic composition, caused in transgenic induction by inserting the gene after the one cell zygotic stage in development.
Outbreeding depression: Phenomenon caused by crossing two organisms that are genetically different where co-adapted gene complexes are broken up and Darwinian fitness reduced.
PCR: (Polymerase Chain Reaction) A technique for amplifying DNA thus producing multiple copies.
Ploidy manipulation: Giving a physical shock that causes the retention of a complete chromosome set that is normally rejected in the production of the egg.
Primer: A short single stranded DNA sequence which when attached by base-pairing to a single stranded template molecule, acts as the start point for complementary strand synthesis directed by a DNA polymerase enzyme.
Promoter sequence: The stretch of DNA upstream of a coding sequence that activates a specific gene.
Reporter gene: A gene which is inserted into the construct to enable detection of the presence of the construct in transgenic cells.
Sex manipulation: Changing the functional sex of a fish by first feeding with an excess of the sex hormone of the other sex.
Southern blotting: A technique for transferring DNA from a gel onto a nylon membrane for subsequent detection (named after E.M. Southern who devised the technique in 1975).
Termination sequence: A DNA sequence which occurs downstream of a functional gene and codes for termination of transcription.

Recent Publications from the Marine Institute

Fisheries Leaflet Series

No. 168	**The Assessment of the Western Mackerel Stock** by John Molloy - Fisheries Research Centre
No. 169	**Fish Kills in Ireland in 1994 and 1995** by Christopher Moriarty - Fisheries Research Centre
No.170	**In preparation**
No. 171	**National activities in the field of aquaculture: Ireland** Edited by David de G Griffith - Fisheries Research Centre

Irish Fisheries Investigations - New Series

No. 1	**The Commercial Exploitation of shrimp** ***Palaemeon serratus*** **(Pennant) in Ireland** by Edward Fahy and Paul Gleeson - Fisheries Research Centre

Fisheries Bulletin

No. 14	**The European eel fishery in 1993 and 1994** Edited by Christopher Moriarty - Fisheries Research Centre

Special Reports

"Towards a Marine Policy for Ireland"
- Proceedings of the Consultative Process.
(September 1996) 182 pp.