FAO Fisheries Circular No. 989 FIRI/C989(E)

ISSN 0429-9329

GENETICALLY MODIFIED ORGANISMS AND AQUACULTURE

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GENETICALLY MODIFIED ORGANISMS AND AQUACULTURE

by
John A. Beardmore and **Joanne S. Porter**
University of Wales Swansea
United Kingdom

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2003

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PREPARATION OF THIS DOCUMENT

This document was written by John A. Beardmore and Joanne S. Porter, under the supervision of Devin Bartley (Inland Water Resources and Aquaculture Service, FAO Fisheries Department), as part of an overall presentation on the role of genetically modified organisms in aquaculture at a special session organized by FAO and the World Aquaculture Society, April 27 2002.

Beardmore, J.A.; Porter, J.S.
Genetically modified organisms and aquaculture.
FAO Fisheries Circular. No. 989. Rome, FAO. 2003. 38p.

ABSTRACT

The production of appropriate genetically modified organisms (GMOs) offers considerable opportunities for more efficient and more effective aquaculture across a wide range of species. Although this potential is being realized in crop production with over 60 million hectares under cultivation, there has been no commercial use of GMOs in aquaculture. Here we review the nature of GMOs, the range of aquatic species in which GMOs have been produced, the methods and target genes employed, the benefits to aquaculture, the problems attached to the use of GMOs and the regulatory and other social frameworks surrounding them. We conclude with a set of recommendations aimed at best practice.

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1. INTRODUCTION

In developing more effective and sustainable exploitation of fish populations, the systematic use of the considerable battery of genetical techniques now available (see e.g. Beardmore, 1998) is still relatively underdeveloped. This statement holds whether we consider natural populations, enhanced populations or cultured stocks. However, there is increasing recognition that combining well established techniques such as the selective breeding programme carried out on Atlantic salmon (Gjoen and Bentsen, 1997) with appropriate molecular techniques, should yield valuable results in aquaculture.

Of the range of molecular techniques available, some may be considered as "platform technologies" following the terminology of Hew and Fletcher (2001), and of these it seems likely that transgenesis will be one of the most significant.

The production of appropriate genetically modified organisms or GMOs (in some cases combined with other forms of genetic improvement) offers considerable opportunities for more efficient and more effective aquaculture across a wide range of species (see for example Sin, 1997). This is likely to be achieved by intervention aimed at removing or reducing current constraints to better production, some of which are listed in Section 3.

The value of GMOs in agronomy is already widely accepted, as the area sown to transgenic crop species world wide exceeds 60 million hectares and this area is increasing rapidly year by year. However, both in terrestrial and aquatic animal species, while many GMOs have been produced, we have not succeeded in obtaining any hard evidence of commercial use. In aquaculture Dunham (1999) has a statement that this is taking place in New Zealand and Scotland though we have no further evidence that this is indeed the case. Carr (1999) refers to small scale production in Cuba though several Cuban scientists have assured one of us (JAB) that there is no commercial production in that country. However, given the drive towards large increases in aquaculture production evident in some countries, e.g. China (Qi Jingfa, 2002) it seems inevitable that commercial production of aquatic GMOs will not be long in coming.

In this paper, our purpose is to discuss, for the benefit of the general community of aquaculturists as well as aquacultural geneticists, the nature of GMOs, the range of aquatic species in which GMOs have been produced, the methods and target genes employed, the benefits to aquaculture, the problems attached to use of GMOs and the regulatory and other social frameworks surrounding them. We conclude with a set of recommendations aimed at best practice.

2. THE NATURE OF GMOS

There are some difficulties in discussing GMOs because of the different definitions employed. At a world level the most recent, and probably the most useful, pronouncement is the so-called Cartagena Protocol on Biosafety to the 1992 Convention on Biological Diversity (SCBD, 2000).

The Protocol does not refer to genetically modified organisms but rather, for reasons that are not explicit, to "living modified organisms" but it is clear that the two terms should be regarded as synonymous.

f) to modify behaviour, e.g. aggression, and
g) to control fertility and/or viability.

While all of these targets are desirable in aquaculture (though to a variable extent depending on the species being considered), work up to now has been focused primarily upon points a. and e.

Melamed *et al*. (2002) provide a useful commentary on some of these applications.

4. GMOS IN AQUATIC SPECIES

The first transgenic animal to be produced was a mouse (Palmiter, Brinster and Hammer, 1982). The first recorded instances of production of transgenics in aquatic species are those of Maclean and Talwar (1984) in rainbow trout and Zhu *et al.* (1985) in goldfish. Since then many species have been used to produce GMOs as shown in Table 1. The list represents an amalgam of species significant in aquaculture with species amenable to laboratory culture and with short life cycles used particularly for studies of gene action, studies which of course form the platform for better understanding and hence better production in aquaculture.

Table 1. Aquatic species in which GMOs have been induced

Common name	**Latin name**	**Number of constructs employed to generate transgenics**
Fish		
Atlantic salmon	*Salmo salar*	6
Coho salmon	*Oncorhynchus kisutch*	4
Chinook salmon	*Oncorhynchus tshawytscha*	3
Tilapia	*Oreochromis spp.*	12
Medaka	*Oryzias latipes*	17
Zebra fish	*Brachydanio rerio*	14
Common carp	*Cyprinus carpio*	14
Channel catfish	*Ictalurus punctatus*	9
African catfish	*Clarias gariepinus*	1
Rainbow trout	*Oncorhyncus mykiss*	7
Cutthroat trout	*Oncorhyncus clarki*	1
Goldfish	*Carrassius auratus*	5
Northern pike	*Esox lucius*	2
Loach	*Misgurnus anguillicaudatus*	2
Sea bream	*Sparus aurata*	2
Red Sea Bream	*Pagrus major*	1
Blunt snout bream	*Megalobrama amblycephala*	1
Nigorobuna	*Carrassius auratus grandoculis*	1
Walleye	*Stizostedion vitreum*	1
Others		
Brine shrimp	*Artemia franciscana*	1
Seaweed	*Laminaria japonica* *Undaria pinnatifida*	1
Sea Urchin	*Strongylocentrotus purpuratus* *Paracentrotus lividus* *Arbacia lixula*	1
Abalone	*Haliotus rufescens*	1

It is clear that Atlantic and coho salmon, tilapia species, catfish, medaka and zebrafish dominate in terms of numbers. Of these fish groups three are very important in aquaculture.

5. THE PROCESS OF GENETIC MODIFICATION

Production of GMOs is a multistage process which can be summarized as follows:

1. identification of the gene interest;
2. isolation of the gene of interest;
3. amplifying the gene to produce many copies;
4. associating the gene with an appropriate promoter and poly A sequence and insertion into plasmids;
5. multiplying the plasmid in bacteria and recovering the cloned construct for injection;
6. transference of the construct into the recipient tissue, usually fertilized eggs;
7. integration of gene into recipient genome; and
8. expression of gene in recipient genome; inheritance of gene through further generations.

5.1 Choice of target genes

As shown in Table 2 the most popular gene used in aquatic species is growth hormone (GH) for reasons that are obvious. GH has been widely used in terrestrial species and as the gene sequence is highly conserved; the product is readily utilized across species boundaries. It may also be noted that, at least in some cases, enhanced growth is associated with more effective utilization of food.

Cold water temperatures are often a major problem in aquaculture in temperate climates when an unusually cold winter can severely damage both production and brood fish stocks of fish. Some marine teleosts have high levels of serum anti-freeze proteins (AFP) or glycoproteins (AFGP) which reduce the freezing temperature by preventing ice-crystal growth. Fletcher, Hew and Davies (2001) have shown that there is one class of AFGP and four classes of AFP. Most are expressed primarily in the liver and some show clear seasonal changes (Melamed *et al*., 2002). Work has particularly focussed on the production of AFP from the winter flounder (*Pleuronectes americanus*), and the gene has been successfully introduced into the genome of Atlantic salmon, integrated into the germ line and passed on to F3 offspring where it was expressed in the liver. However, a number of Ala, Pro-specific endopeptidases are required for production of mature proteins and these are not present in Atlantic salmon. Furthermore, the AFP gene in winter flounder, and possibly other Arctic species, exists in many copies (see Section 7). Thus, much further work is required in order to develop effective antifreeze activity in Atlantic salmon (Hew *et al*., 1999). Work on AFP has also been conducted in goldfish (Wang *et al*., 1995) and milkfish (Wu *et al*., 1998).

Genetic manipulation has also been undertaken in order to increase the resistance of fish to pathogens. This is currently being addressed by the use of DNA vaccines (encoding part of the pathogen genome) and antimicrobial agents such as lysozyme (Demers and Bayne 1997). An example is the injection of Atlantic salmon with a DNA sequence encoding infectious hematopoeitic necrovirus (IHNV) glycoprotein under the control of the cytomegalovirus promoter (pCMV). Challenge with the virus eight weeks later revealed that a significant degree of resistance had been achieved. The fish were still resistant and were shown to have

Table 2. Results in induction of GMOs in aquatic species.

Species	Target gene	Int	Exp	Trans	Reference
At. Salmon	GH	+	+	+	Hew & Fletcher, 2001
At. Salmon	AFP	+	+	+	Hew & Fletcher, 2001
Coho salmon	GH	+	+	Nd	Stevens Devlin, 2000
Tilapia	tiGH	+	+	+	Martinez *et al.,* 1999
Tilapia	Fish GH	+	+	Nd	Rahman & Maclean, 1999
Carp	GH	+	+	Nd	Hinits & Moav, 1999
Salmon	Glucose transporter and hexokinase	+	+	+	Pitkanen *et al*., 1999
Tilapia	GH	+	+	Nd	Rahman *et al*., 1998
At. salmon	GH	+	+	+	Saunders, Fletcher & Hew, 1998
Carp	HGH	+	+	+	Fu *et al.,* 1998
At. salmon	GH	+	+	+	Stevens, Sutterlin & Cook, 1998
Tilapia	tiGH	+	+	+	de la Fuente *et al*., 1998
Tilapia	INT-tiGH	+	+	+	de la Fuente *et al*.,1998
Tilapia	CSGH	+	+	+	Rahman & Maclean, 1998
Tilapia	INT-tiGH	+	+	+	Hernandez *et al*., 1997
Tilapia	ypGH	+	+	Nd	Chen *et al*., 1997
Abalone	GH	+	+	Nd	Powers, Kirby & Gomez-Chiarri, 1996
Medaka	CAT	+	+	+	Kinoshita *et al*., 1996
Tilapia	GH	+	+	+	de la Fuente *et al*., 1996
At. salmon	GH AFP	+	+	+	Choy *et al*., 1996
Tilapia	tiGH	+	+	+	Martinez *et al*., 1996
Tilapia	Lac Z	+	+	+	Alam *et al*., 1996
Tilapia	tiGH	+	Nd	Nd	Martinez *et al*., 1996
Coho salmon	GH	+	+	+	Devlin *et al*., 1995a
Pacific salmon	CSGH	+	+	+	Devlin *et al*., 1995b
Common carp	RTGH	+	+	Nd	Chatakondi *et al*., 1995
Common carp	CSGH	+	+	+	Moav *et al*., 1995
Medaka	Lac Z	+	+	Nd	Tsai, Tseng & Liao, 1995
Brine shrimp	Luciferase reporter gene	+	Nd	Nd	Gendreau *et al*., 1995
Common carp	RTGH	+	+	Nd	Chatakondi *et al*., 1995
Common carp	CSGH	+	+	+	Moav *et al*., 1995
Pacific salmon	CSGH	+	+	+	Devlin *et al*., 1995b
Rainbow trout	CSGH	+	+	+	Devlin *et al*., 1995a
Cutthroat trout	CSGH	+	+	+	Devlin *et al*., 1995b
Chinook salmon	CSGH	+	+	+	Devlin *et al*., 1995b
Loach	CSGH	+	+	Nd	Tsai, Tseng & Liao, 1995
Salmon	GH	+	+	Nd	Devlin *et al*., 1994
Chinook salmon		+	Nd	Nd	Sin *et al*., 1994
Laminaria japonica	Plasmid BI221	+	+	Nd	Qin *et al*., 1994
Undaria pinnatifida	Plasmid BI221	+	+	Nd	Qin *et al*., 1994
Nigorobuna	*E. coli* beta galactosidase	+	+	Nd	Ueno *et al*., 1994
Blunt snout bream	HGH	+	+	+	Wu *et al*., 1994
Common carp	HGH	+	+	+	Wu *et al*., 1994
Oreochromis niloticus	Bacterial lacZ	+	+	+	Mclean, 1994
Zebrafish		+	Nd	Nd	Hackett *et al*., 1994
African catfish	AFP GH	+	+	Nd	Erdelyi *et al*., 1994
Common carp	AFP GH	+	+	Nd	Erdelyi *et al*., 1994
Abalone	GH	+	+	Nd	Powers *et al*., 1994
Pacific salmon	GH sockeye salmon	+	+	Nd	Devlin *et al*., 1994
Zebrafish	Firefly luciferase	+	+	–	Patil, Wong & Khoo, 1994
Zebrafish	CSGH	+	+	+	Zhao, Zhang & Wong, 1993
Common carp	RTGH	+	+	+	Chen *et al*., 1993

Species	Target gene	Int	Exp	Trans	Reference
Zebrafish	luciferase	+	–	-	Kavumpurath *et al*., 1993
Common carp	HGH	+	+	+	Cui *et al*., 1993
Tilapia	RGH	+	Nd	Nd	Rahman & Maclean, 1991
Zebra fish	CAT	+	Nd	Nd	Khoo *et al*., 1992
Tilapia	HGH	Nd	Nd	Nd	Ber *et al*., 1992
Zebrafish	CAT	+	+	Nd	Sharps *et al*., 1992
Goldfish	Neomycin resistance CAT	+	+	Nd	Guise, Hackett & Faras, 1992
Northern Pike	BGH	+	+	Nd	Guise, Hackett & Faras, 1992
Atlantic salmon	Winter flounder AFP	+	+	+	Fletcher, Davies & Hew, 1992
At. salmon	Bacterial CAT Chinook salmon GH	+	Nd	Nd	Jun Du *et al*., 1992
Common carp	RTGH	+	+	+	Chen *et al*., 1992
Channel catfish	RTGH	+	+	+	Chen *et al*., 1992
Northern Pike	Bacterial CAT BGH and CGH	+	Nd	Nd	Moav *et al*., 1992
Walleye	Bacterial CAT BGH and CGH	+	Nd	Nd	Moav *et al*., 1992
Zebrafish	Bacterial CAT BGH and CGH	+	Nd	Nd	Moav *et al*., 1992
Carp	Grass carp GH	+	Nd	Nd	Zhu, 1992
Zebrafish		+	–	Nd	Khoo *et al*., 1992
Northern pike	BGH CSGH	+	+	Nd	Gross *et al*., 1992
Channel catfish	Salmon GH	+	+	Nd	Dunham *et al*., 1992
At. salmon	CSGH	+	+	Nd	Jun Du *et al*., 1992
Gilthead seabream	BGH and HGH	+	–	Nd	Cavari *et al*., 1993
Rainbow trout	Carp alpha globin	+	+	+	Yoshizaki *et al*., 1991
Rainbow trout	BGH	+	+	Nd	Chandler *et al*., 1990
Tilapia	HGH	+	Nd	Nd	Brem *et al*., 1988

Abbreviations used in Table 2 above :

At:	Atlantic salmon
GH:	Growth Hormone
AFP:	Anti-freeze Protein
Nd:	Not determined
HGH:	Human Growth Hormone
BGH:	Bovine Growth Hormone
CS:	Coho Salmon Growth Hormone
YP:	Yellowfin Porgy Growth Hormone
CAT:	Chloramphenicol Acetyl Transferase
TiGH:	Tilapia Growth Hormone
RTGH:	Rainbow Trout Growth Hormone
RGH:	Rat Growth Hormone
Int:	Integration
Exp:	Expression
Trans:	Transmission

generated antibodies three months later (Traxler *et al*., 1999). Similar studies have been undertaken for other fish diseases eg. Haemorrhagic septicaemia virus (VHS) (Lorenzen, Olesen and Koch, 1999) and work of this kind appears to have great potential value for fish farms (Melamed *et al*., 2002). We would also draw attention to the work using a cecropin B gene from the moth *Hyaloplova cecropin.* When channel catfish transgenic for this gene were challenged with *Flavobacterium columnare* and *Edwardsiella ictaluri* survival was better for transgenics than controls (Dunham *et al*., 2002)

There appears to be no published evidence for integration of vaccine DNA into the recipient genome. Nevertheless, the persistence of the DNA appears often to be relatively long which suggests some replication (not normally expected with non-chromosomal pieces of DNA). It seems desirable for the moment to regard such treated animals as "transient" GMOs rather than full GMOs.

5.2 Isolation of the gene of interest

Usually the gene of interest will already be available as an element of a "library" of short sections of the total genome of the donor strain or species. If this is the case the procedure followed is to multiply the gene using the PCR reaction. If, however, the gene is to be taken from a genome not previously investigated, a more complex procedure will need to be followed. The use of the technique of the Polymerase Chain Reaction (PCR) enables the gene in both the cases noted above to be multiplied to the level of several million copies needed for the generation of the construct (see Section 5.3).

5.3 Cloning the gene of interest

When many copies of the target gene have been generated, the gene is placed in a "construct" (see Section 5.4). Once the gene of interest has been ligated enzymatically into the construct, this whole complex is ligated into bacterial plasmids (see Figure 3), which act as "production vectors" and enable the gene to be replicated many times within the bacterial cells. The

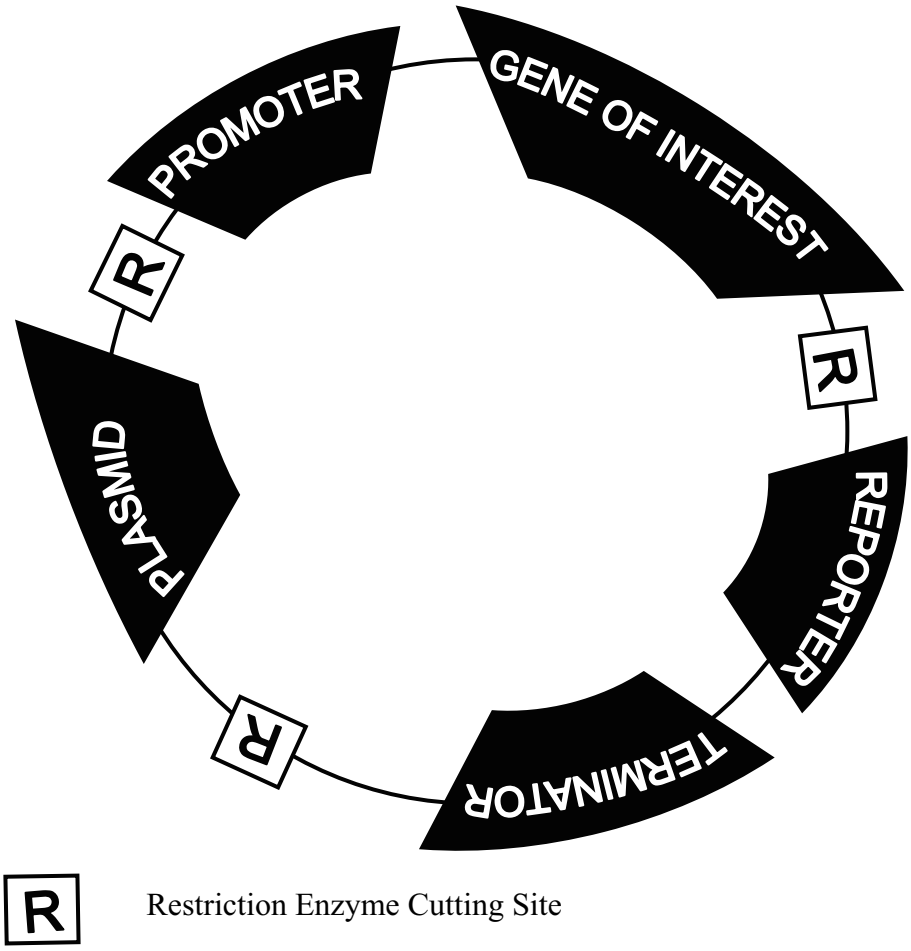
bacteria are then plated out. It is possible to tell from reporter genes (see below) whether the vector has been taken up by the bacterial cells. This usually involves some colour change in the colonies containing inserted DNA. The many times amplified DNA construct is then enzymatically cut out of the plasmids (after these have been removed from the bacterial cells) and it is ready to be used for insertion into eggs of the host species.

A more detailed outline of the technical details of the processes outlined in Sections 5.2 and 5.3 may be found in Maclean (1998).

5.4 The construct

A construct is a piece of DNA which functions as the vehicle or vector carrying the target gene into the recipient organism. It has several different regions as shown in Figure 2. There is a promoter region which controls the activity of the target gene, a region where the target DNA is inserted, usually some type of reporter gene to enable one to ascertain whether the target has combined successfully with the construct and a termination sequence.

Figure 2.
Diagram of DNA sequence of a basic plasmid and incorporated construct.



The sources of these several DNA sequences may be different species although promoter and target genes would ideally be derived from the same species

As shown in Table 3, constructs have been reported from 92 studies. The number of different constructs is greater than the number of target genes used in aquaculture and a substantial research effort has been made in this area. From the early 1990s research focussed on developing "all fish" constructs in preference to using mammalian promoters.

The use of all-fish constructs has dramatic effects on expression of transgenes, e.g. Devlin *et al*. (1994), developed an all salmon gene construct which accelerates the growth of transgenic salmonids by over 11 fold. In tilapia, Maclean (1994) found that using carp beta actin instead of rat beta actin promoter led to a ten fold increase in production of hormone in transgenic animals.

Table 3. Summary of major research effort in inducing GMOs in aquatic species.

Species	Target gene	Typical construct	Typical induction method	Number of studies
Salmon spp.	GH AFP	Ocean pout AFP linearized DNA	Microinjection	17/92
Rainbow Trout	GH	Ocean pout AFP	Microinjection	14/92
Tilapia spp.	GH	Cytomegalovirus (CMV)	Microinjection	12/92
Carp	GH	Rous Sarcoma Virus Long Tandem Repeat	Microinjection	17/92
Zebrafish	Luciferase	pMTL plasmid	Microinjection	16/92
Medaka	CAT	AFP	Microinjection	11/92

Other important work suggested that the optimal stage at which the transgene is introduced might vary between cells and species eg. Garcia del Barco *et al.* (1994) using Zebrafish showed that there were differences in the regulatory requirements for cells and embryos, and suggested therefore that constructs should be assayed in both cells and embryos.

Other work shows how critical the nature of the gene construct is. Devlin *et al.* (1995a) showed that using an opAFPGHc gene construct in coho salmon eggs gave rise to some alevins which had the typical brown colouration, while the remainder displayed a distinct green colouration. The results suggest that the green phenotype arose from the presence of the opAFPGHc construct and therefore could be indicative of transgene uptake/transmission. All the offspring were tested by PCR for presence of the transgene and 182 of 184 alevins were correctly assigned on this basis. However, it was found that later in development all fish turned green (the normal colour later in development) and so the transgenic fish were showing accelerated growth. Later in development it was found that most of the transgenic fish showed signs of cranial abnormality probably due to accelerated growth (see Section 9.3). While the onstruct was useful in that transgene uptake could be monitored, further work was needed to ensure that healthy fish could be produced.

5.5 Techniques for inducing transgenics

Transgenic fish have largely been produced through microinjection into fertilised eggs or early embryos (see Table 2). Electroporation of sperm has been shown to be successful in some species eg. Zebrafish (Khoo *et al.,* 1992) Chinook salmon (Sin *et al*., 1994) and Loach (Tsai, Tseng and Liao, 1995). Liposomes have also been utilized as vectors (Khoo 1995). Ballistic methods using microprojectiles have been investigated in *Artemia* with a view to their use in generating transgenic crustacea (Gendreau *et al*., 1995) and also in seaweed species (Qin *et al*.,1994). "Baekonisation", an electric, flat field type of electroporation was utilized to transfer DNA into Zebrafish embryos (Zhao, Zhang and Wong, 1993), this method appeared to be successful but has not been taken up in the same way as other forms of electroporation and microinjection methods.

More recently the use of embryonic stem cells (ESC) as a method for inducing transgenesis has been advocated. These cells are undifferentiated and remain totipotent, so they can be manipulated in vitro and subsequently reintroduced into early embryos where they can contribute to the germ line of the host. In this way genes could be stably introduced or deleted (Melamed *et al.*, 2002). Despite the early success of ESC technology in mice, the uptake of the technology for fish has been slow, although early precursor cells (Mes 1) have been cultivated from Medaka and show many of the same features as mouse ESC. Studies by Hong, Winkler and Schartl (1996, 1998) and Hong, Chen and Schartl (2000) showed that 90 percent of host cell blastulae transplanted with Mes 1 cells developed into mosaic fry, and these cells became integrated into organs derived from all three germ layers, and differentiated into various types of functional cells.

Another example of new and possibly more efficient ways for gene transfer is the use of pantropic retroviral vectors. These are able to infect a wide range of host cells and have been used to infect newly fertilized Medaka eggs with a reporter gene, which appeared to become integrated into the entire germ line of some of the P1 females (Lu, Burns and Chen, 1997). In Zebrafish when retroviral infection and microinjection were compared, the two methods were equally efficient in passing the transgene into eggs, but there was wider variability in the extent of reporter gene expression among those founders that were microinjected (Linney *et al*.,1999). However, the use of retroviruses is not without problems (see Section 9.1).

The microinjection method is suitable for relatively small numbers of organisms being manipulated whereas electroporation, sperm/liposome mediation and bombardment methods are more suitable for mass treatments. The most popular method of insertion of transgenes in aquaculture is microinjection; in 92 studies reviewed from 1985 to the present, 68 used microinjection, eleven used sperm mediated methods, six used electroporation and five used both sperm mediation and electroporation. However, the problem of mosaic expression of the transgenes is common, and this gives rise to varying proportions of transgenic genotypes in the progeny.

A useful review of technical details of the techniques mentioned can be found in Sin (1997).

5.6 Integration sites

The factors determining sites of integration are still poorly understood though research in this direction is increasing. It is particularly important to gain greater accuracy in controlled site of integration because of the unpredictable effects of uncontrolled integration on resident genes. Caldovic and Hackett (1995) tested the ability of special sequences called transposable border elements from other species to confer position-independent expression of transgenes or enhance integration of transgenic constructs into fish chromosomes. Early results indicate that such elements from some species do not act as enhancers and do not improve integration frequencies. However, both avian and insect border elements were found to confer position-independent expression as judged from expression of CAT genes in F_1 fish. Hackett *et al.,* (1994) showed that co-transfer of retroviral integrase protein with transgenic DNA can accelerate and enhance the rate of integration. More studies of this type are needed to improve the success and controlled positioning of integration of transgenes in the future.

5.7 Expression of gene

The uptake and integration of a transgene does not guarantee that the gene will express itself in the new genetic environment. Tests must be carried out to determine whether there is

expression and if there is expression, at what level this takes place. Clearly, in commercial aquaculture only those transgencs expressing the target gene at a sufficiently high level will be of interest.

5.8 Inheritance of gene

A fish which expresses the target gene at an acceptable level may not be able to transmit the gene to progeny. This is because many transgenics are mosaic individuals and unless the gonads are included in the tissues possessing the transgene the transgenic animals will not breed true. Appropriate breeding tests must, therefore, be carried out.

The high proportion of mosaic individuals is one reason why the proportions of progenies of different genotypes resulting from parents that are putatively hemizygous for a transgene do not necessarily conform to mendelian expectations. Another reason is the integration of two or more copies of the transgene at different sites in the recipient genome. Further breeding tests will be required in order to establish a pure breeding line of transgenic fish.

6. FIELD OF TRANSGENICS

Inducing transgenics is a relatively inefficient process. According to Maclean (in press) relevant variables include the species, the workers involved and presumably also the techniques. For every hundred eggs injected, a yield of about ten percent of fish testing positive for the presence of the transgene may be expected. However, only about one percent of the eggs treated will prove ultimately to be germ line positive and capable of transmission to the next generation. This figure is in line with that found for pigs, sheep, goats and cattle (Royal Society, 2001).

7. GENETIC ARCHITECTURE OF QUANTITATIVE CHARACTERS

Most of the phenotypic characters of interest to aquaculture are quantitative rather than qualitative. It is therefore important to understand the genetic architecture of such characters. The polygenic theory of quantitative characters (developed by Mather, 1943) envisaged a fairly large number of loci each with relatively small and equal effects acting in a largely additive way on a quantitative character. The theory made no assumptions about the heritability of the character so that two discrete characters might have similar numbers of loci concerned in their determination but very different values for genetic and environmental variance when these were partitioned from the total phenotypic variance. Over the years it has indeed been observed that relatively large numbers of loci (of the order of 50 according to the work of Shrimpton and Robertson 1988a and b) may be involved but also that effects of dominance and epistasis are frequently involved and that the magnitude of the effect produced by each locus can vary considerably (Mather, 1979).

Current informed thinking on genetic architecture is admirably described by Falconer and Mackay (1996). It can be summarised as follows:

A typical quantitative character is likely to involve:

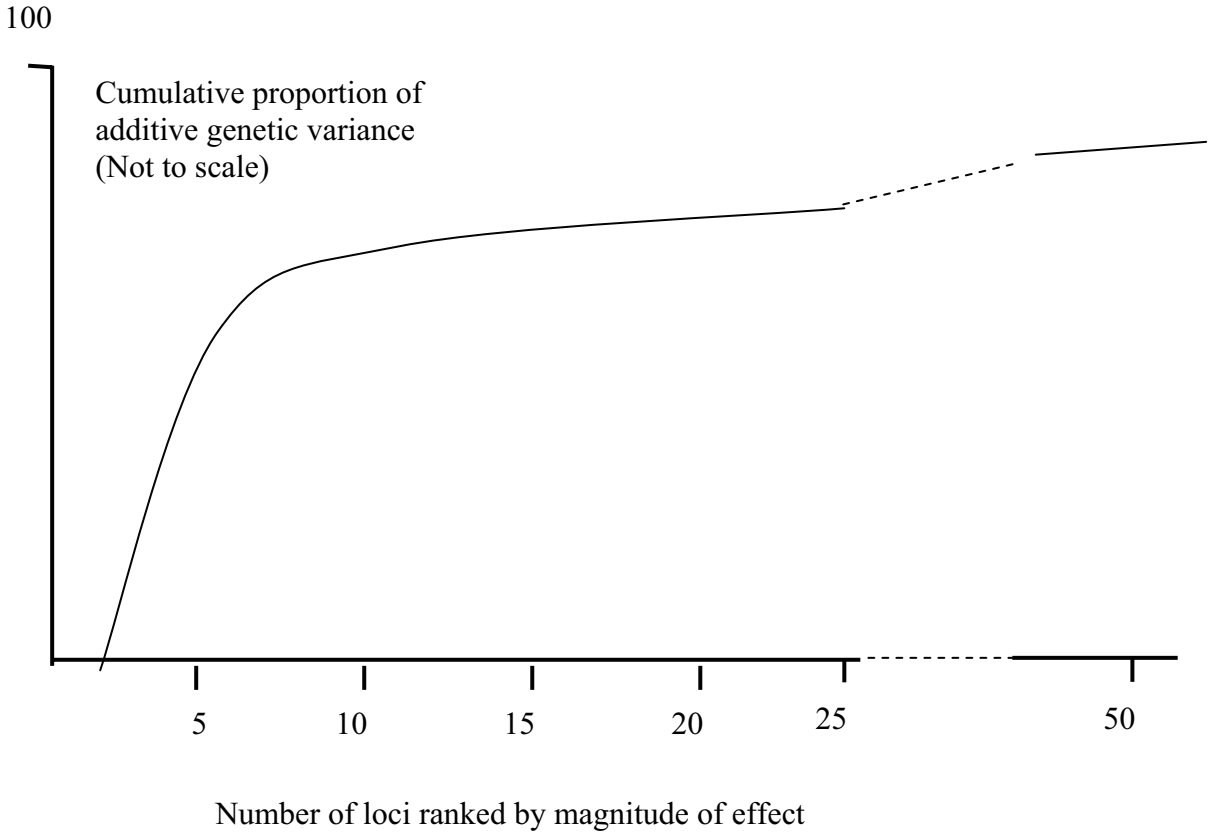
1. a number of loci which may reach several tens in number;

2. genes acting in ways which may be additive, dominant, epistatic and interactive with environmental factors; and

3. considerable variation in individual locus effect (including many small effects from genes whose primary effect is elsewhere on the phenotype through pleiotropic effects).

The last point is quite important as it suggests that typically a small number of loci account for a very large fraction of the variation in the character (see Figure 3).

Figure 3.
Graphical theoretical representation of the relationship between the number of loci determining a typical character and the cumulative proportion of the additive genetic variance account for by such loci.



The work of Devlin *et al.* (2001), which suggests that the benefits of transgenic technology in strains already subject to selection for the desired phenotype may be negligible, is relevant here. The selected strain of rainbow trout which they used might reasonably be assumed to be homozygous for favourable alleles at most of the loci of larger effect.

These observations lead inescapably to the conclusion that success in achieving the desired phenotype in transgenic animals will depend on the nature of the genetic architecture of the character concerned. Where gene action is largely additive more progress may be expected than where more complex aspects of gene action are seen. However, where gene action is additive success may still be disappointing as with the transgenic AFP referred to in Section 5.1. Hayes, Davies and Fletcher (1991) have shown that the AFP gene exists in multiple copies in the genome of the winter flounder *Pseudopleuronectes amenis* and that the number

may be as high as 40. Thus, to produce successful AFP phenotypes through transgenesis is a highly complex and demanding objective as the effects of individual loci in this case are probably roughly equal.

8. BENEFITS ARISING FROM THE USE OF GMOS

8.1 Aquaculture

Evidence of real benefit in terms of economically significant characters comes mainly from work on growth hormone (GH) (Table 4). The overall conclusion from the studies of several workers is that fish GH transgenics enjoy growth rates markedly superior to those in comparable (in some cases sibling) non transgenics. Studies have revealed enhancement of growth particularly in salmonids to an average of 3−5 times the size of non-transgenic controls with some individuals reaching as much as 10−30 times the size of controls (Devlin *et al*., 1994). The economic gains to be made from use of such GMOs are obvious and transgenics must therefore be considered as a route for providing superior strains along with selective breeding (Melamed *et* al., 2002). We should note that, not surprisingly, lines resulting from different transgenic events with the same construct in the same population may give different results and this has been confirmed in field trials (Dunham *et al.*, 1992).

Table 4. Actual and potential benefits of GMOs to aquaculture.

Species	Genetic modification	Potential benefit	Actual benefit	Reference
Atlantic salmon	GH and AFP	To enhance growth and increase cold tolerance	Enhanced growth and increased tolerance to cold	Melamed *et al*., 2002
Mud loach	Triploidy	To induce sterility	Accelerated growth, gigantism and likely sterility	Nam, Cho & Cho, 2001
Atlantic salmon	AFP	Increase low temperature tolerance	Precursor AFP has only 70% activity of AFP. AFP promoter has potential as a construct for transgenic studies.	Hew & Fletcher, 2001
Carp	GH	To enhance growth	Higher growth rates than the non-transgenic controls	Hinits and Moav, 1999
Tilapia	GH	To enhance growth	Stable germ line transmission in a fast growing transgenic line	Martinez *et al*., 1999
Rainbow trout and Arctic charr	Glucose transporter and hexokinase genes	To evaluate possibility of improving carbohydrate metabolism efficiency of salmonid fish	Some positive results in first generation	Pitkanen *et al*., 1999
Tilapia	GH	To enhance growth	Up to 30 times > than non-transgenics	Rahman & Maclean, 1999
Tilapia	GH	To enhance growth	Homozygous transgenic fish produced, growth enhanced, fertility reduced	Rahman *et al*., 1998

Species	Genetic modification	Potential benefit	Actual benefit	Reference
Seabass	DNA Vaccine	To manage viral diseases in farmed fish	Foreign gene transferred by injection into the muscles	Sulaiman, 1998
Atlantic salmon	GH	Transgenic fish may have different respiratory and swimming performance than non-transgenics	Oxygen demand of transgenics 1.6 times higher than non-transgenics. Swimming speed no different.	Stevens, Sutterlin & Cook, 1998
Tilapia	GH	To enhance growth	Up to 30 times > than non-transgenics	de la Fuente *et al*., 1998
Tilapia	YPGH	To enhance growth	Transgenics heavier and grew faster than non-transgenics	Chen *et al*., 1997
Zebrafish	Triploidy induction	To induce sterility	Expression confirmed	Marichamy, 1997
Tilapia	GH	To enhance growth		Hernandez *et al*., 1997
Tilapia	GH	To enhance growth	Up to 30 times > than non-transgenics	Martinez *et al*., 1996
Rainbow trout	GH	To enhance growth	Significant growth enhancement	Chen *et al*., 1996
Atlantic salmon	GH	To enhance growth	Growth enhancement	Hew *et al*., 1996
	AFP	To increase low temperature tolerance		
Coho salmon	GH	To enhance growth	>10 fold increase in size of transgenic fish	Devlin *et al*., 1995a
Carp	GH	To enhance growth	32-87% inheritance when transgenic parents crossed. 0-50% inheritance when transgenic and non transgenic fish mated.	Moav *et al*., 1995
Carp	GH	To enhance growth	Body composition was altered; % fat, % moisture content was lower for transgenics and amino acid ratios were altered.	Chatakondi *et al*., 1995
Carp	Transfer of border elements	To confer position independent expression of transgenes or enhance integration	Confer position independent expression	Caldovic & Hackett, 1995
Medaka	Lac Z gene	To initiate lacZ gene expression in embryos	Gene expression initiated at midblastula stage	Tsai *et al*., 1995
Zebrafish	Cotransfer of retroviral integrase protein with transgenes	To accelerate and enhance rate of integration of transgene	Enhances and accelerates rates of integration	Hackett *et al*., 1994

Species	Genetic modification	Potential benefit	Actual benefit	Reference
Salmon	GH with all salmon construct	To enhance growth	Accelerates growth by over 11 fold	Devlin *et al*., 1994
Catfish and carp	Coinjection of reporter gene with GH gene	To enhance integration	Rate of cointegration higher than expected for independent events	Erdelyi *et al*., 1994
Tilapia	GH	To enhance growth	Growth enhancement in F1 animals	Martinez *et al*., 1994
Zebrafish	Luciferase gene	Use of luciferase as a reporter of expression	Method compared favourably with southern blotting and PCR.	Patil, Wong & Khoo, 1994
Tilapia	Lac Z gene	To report on expression levels	Expression of reporter gene indicated that carp promoter was 10 times more efficient than rat promoter	Maclean, 1994
Trout	Chromosome manipulation and monosex production	To increase production	Increased production	Stein, 1993
General	Disease resistance genes	To develop disease resistant lines		Fjalestad , Gjedrem & Gjerde, 1993
Zebrafish	Luciferase gene	Use of luciferase as a reporter of expression	Stable integration of luciferase	Kavumpurath *et al*., 1993
Gilthead seabream	GH	To enhance growth	Growth enhanced by 20% after two weeks	Cavari *et al*., 1993
Carp	GH	To enhance growth	Significant but variable	Chen *et al*., 1993
Zebrafish	Promoter activity	To enhance integration	Human cytomegalovirus gave best results	Sharps *et al*., 1992
Channel catfish	GH	To enhance growth	20% larger than non-transgenic siblings	Chen *et al*., 1992
Goldfish and northern Pike	Neomycin resistance, CAT and GH	To assess applicability of neomycin resistance as a marker in piscine systems	Preliminary results showed transfer and expression.	Guise, Hackett & Faras, 1992
Atlantic salmon	AFP	To enhance cold resistance	Establishment of stable transgenic lines of Atlantic salmon	Fletcher, Davies & Hew, 1992
Atlantic salmon	GH	To enhance growth	9/450 positive fingerlings identified by PCR analysis	Jun Du *et al*., 1992
Rainbow trout	GH	To enhance growth	A significant fraction of the F1 inherited the gene, and these grew faster than non-transgenic siblings.	Chen *et al*., 1992
Atlantic salmon	GH and AFP	To enhance growth and increase cold tolerance	Transgenic fish grow on average four times faster than non-transgenics	Fletcher, Davies & Hew, 1992

Species	Genetic modification	Potential benefit	Actual benefit	Reference
Atlantic salmon	GH	To enhance growth	At one year old transgenic fish were 2 to 6 fold larger than non-transgenic siblings	Jun Du *et al*., 1992
Channel catfish	GH	To enhance growth	F1 transgenic progeny grew 26% faster and 40-50g heavier than non-transgenic siblings	Dunham *et al*., 1992
Rainbow trout	Carp alpha globin		7/30 progeny from one of the transgenic males carried the alpha globin gene. 1 of this seven had 50 copies integrated into the genome	Yoshizaki *et al*., 1991
Medaka	AFP	To increase cold tolerance		Gong, Vielkind & Hew, 1991
Atlantic salmon	AFP	To increase cold tolerance	24/137 progeny carried the AFP gene	Shears *et al*., 1991
Goldfish	Neomycin resistance gene	To assess use of gene as a marker for expression	Successful in one fish	Yoon *et al*., 1990
Carp	GH	To enhance growth	20/365 showed integration and expression	Zhang *et al*., 1990
Rainbow trout	Chromosome mediated gene transfer	Generations of transgenics	Success was variable depending on female used	Disney, 1989
Atlantic aalmon	AFP	To increase cold tolerance	Stable integration and a low level of expression	Shears *et al*., 1989
Carp and loach	GH	To enhance growth	A significant fraction of the F1 progeny inherited the foreign gene	Chen & Powers, 1988
Carp	GH	To enhance growth	20/380 fish were found to contain introduced gene.	Zhang *et al*., 1988
Zebrafish and rainbow trout	Reporter genes; neomycin transferase, CAT and beta galactosidase	To assess use of them in detection of expression of transgenes	Reporter genes could prove useful	Gibbs, Gray & Thorgaard, 1988
Tilapia	GH	To enhance growth	Integration rate is lower than in mammals	Brem *et al*., 1988

The species involved include Atlantic salmon (Du *et al*., 1992), coho salmon (Devlin *et al.*, 1995a), Nile tilapia (Rahman *et al*., 1998) and interspecific hybrid tilapia (Martinez, 1996). Work reported on carp (Chatakondi *et al*., 1995) and channel catfish (Dunham, 1996) shows less but still significant effect but, as indicated by Maclean and Laight (2000), this may be a consequence of 1) choice of promoter sequence and 2) a background of selective breeding in the strain used. In most cases the transgenics will be hemizygous for an unknown number of copies (possibly often one) of the transgene.

There is a most interesting suggestion from the work of Martinez *et al.* (1999) using tilapia GH in *O. hornorum urolepsis* that fish hemizygous for the transgene are superior in growth rate not only to wild type sibs, but also to transgenic homozygotes. This, if a real and general effect, may be of considerable significance for the use of GH transgenics in aquaculture and the maintenance of broodstock.

Considerable interest exists in making fish transgenic for the antifreeze protein genes found in some species such as winter flounder and if the difficulties involved in securing phenotypic expression of the antifreeze phenotype in a phenotype controlled by multiple loci can be solved (Hew *et al*., 1999; Hayes, Davies and Fletcher, 1991), the benefits would be very large.

There are also a number of other target phenotypes for which transgenics offer considerable potential. These include salinity tolerance, sterility, control of sexual phenotype, disease resistance to specific pathogens (Mialhe *et* al., 1995) and behavioural modifications. One particularly interesting possibility is that of modifying the genome to allow greater production of omega-3 fatty acids (Donaldson, 1997). There are, as yet, few concrete data which can be reported but clearly there are very promising areas of work which could bring substantial benefits to aquaculture.

The introduction of a transgene is intrinsically unlikely to have only one effect on the phenotype and possible pleiotropic effects need to be considered. These could in principle, be of two kinds:

i) genuine pleiotropy manifested through, for example, dose effects in the metabolic network; and

ii) apparent pleiotropy arising from disturbance in functioning of resident genes through integration of a transgene at a specific point in the genome. Such disturbances might be favourable or unfavourable.

It will not always be easy to distinguish between genuine and apparent pleiotropy. However, Chatakondi *et al*., (1995) and Dunham (1996) have reported favourable effects such as increased carcass yield, increased protein level, reduced fat and greater tolerance of low dissolved oxygen levels in common carp and channel catfish transgenic for rainbow trout GH. Dunham (1999) has argued, without an explicit rationale, that "disease resistance will likely be improved directly".

Possible effects of other elements in the construct such as reporter genes or antibiotic resistance genes need mention. Such cotransgenes confer no benefits and may pose significant risks (particularly with antibiotic resistance genes). Best practice would certainly require removal of such elements before commercial use of the target transgenes is started (MAFF, 1994).

8.2 Other uses of transgenics in aquatic species

While the primary focus of this paper is on uses of transgenics is in improving production in aquaculture, it is worthwhile pointing out that there are several other potential uses with strong connections to aquaculture. These include living pollution monitors achieved by incorporating a pollution sensitive promoter in the transgenic animal.

A typical example would be a green fluorescent protein structural gene (GFP) driven by a metallothionin promoter. If the promoter is inactivated by heavy metal pollution the GFP is switched off and the colour change is readily visible. Another use closely related to aquaculture, is that of using fish as a production system for valuable gene products which can be extracted in a comparable fashion to similar production in mammalian species. Such products might include vitamins and work is underway to produce factor VII (one of the human blood clotting factors), in tilapia (Maclean, 2002).

Use of AFP of a tangential kind includes cases where it has been used to help protect membranes from cold and freezing damage by modification of the structure of membranes *in vitro* (Rubinsky *et al*., 1992; Rubinsky, Arav and DeVries, 1992). The ability of fish AFP's to preserve sheep and pig embryos has been demonstrated (Arav *et al*., 1993; Baguisi *et al*., 1997). The use of AFP's in cryopreservation of fish eggs and embryos still awaits further development (Melamed *et al*., 2002). However, some initial work has been carried out by (Lubzens, Rothbard and Hadani, 1993) who were able to cryopreserve spermatozoa from the ornamental Japanese carp (nishikigoi). Work exploiting AFPs generated by transgenic fish could become most useful in hatcheries in future in order to preserve transgenic lines and to supply new hatcheries and farms with suitable stocks.

8.3 Commercial significance

The demand for fish is increasing year on year and the yield from capture fisheries is declining. Thus, although aquaculture production is increasing the market for further expansion in aquacultural production is likely to be very good for many years to come.

An OECD (1995) view was that the time scale from 1995 for GMOs in salmon to be commercialized would be 15 years and that for tilapia would be five years. As matters stand at present the estimates for both species would lie between the two figures given. It is reported (Stokstad, 2002) that Atlantic salmon transgenic for a Chinook salmon GH gene are being considered for approval in aquaculture in the USA.

The data available on GH transgenics suggest that the monetary benefits to be obtained from use of these fish will be large. For comparison, the use of the single step genetic change represented by monosex genetically male tilapia (GMT) in Nile tilapia (though this is not a GMO) increased production by almost 30 percent and effectively doubled the net income, from this source, of Philippine farmers growing it (Mair *et al*., 1995; Mair and Abella, 1997). Nevertheless it is sensible to recognize that the benefits of use of GMOs are not always clear cut, at least in crop plants in the USA (Soil Association, 2002).

9. RISK FACTORS OF GMOS

There are a number of publications which address this issue. Maclean and Laight (2001) and Dunham (1999) have produced very useful reviews which discuss many of the points raised in this paper.

In our view the most important areas of risks which need to be considered in the use of transgenics are:

1. human health
2. biodiversity
3. animal welfare
4. poor communities

In each of these categories there exists a multiplicity of pathways by which effects could, in principle, be brought about. Rational and responsible assessment of risk requires that the following properties are all considered:

1. source of the DNA of the target gene;
2. source of the non target DNA segments of the construct used;
3. site(s) of incorporation of the transgene within the recipient genome;
4. product of the transgene;
5. interaction of the transgenic product with other molecules in host and consumer;
6. possible molecular changes in transgene product during processing;
7. pleiotropic effects of transgene;
8. tissue specificity of transgenic expression; and
9. numbers of transgenic organisms capable of interacting with natural systems).

9.1 Human health

The risks to health will depend upon all of the factors listed above. In practical terms the most important of these are likely to be the source of the DNA and the nature of the product.

The great majority (98 percent) of dietary DNA is degraded by digestive enzymes relatively quickly (Royal Society, 2001) but use of viruses (disarmed or otherwise) as vectors, must increase the risk factor significantly as these are organisms which are adapted to integrating into host genomes and some represent risk factors for cancer induction. The work of Zhixong Li *et al. (*2002) who induced leukaemia by using retroviral vectors in making transgenics for a commonly used marker gene in mice and a recent report of leukaemia induction in a child undergoing gene therapy for x-SCID using a retrovirus (Hawkes, 2002) show that this is not a trivial risk. Arguments about risks and benefits attached to this form of gene therapy are current (Kaiser, 2003).

At the other extreme the use of autotransgenics must be seen as posing a risk which is orders of magnitude lower than that for allotransgenics and probably negligible. The major risk

from the production of the transgene will lie in the use of novel proteins or other molecules produced by the transgenic organisms. Either in the native form or, following modifications in the human body, such molecules could be inimical to human health (e.g. through allergies). It would seem sensible to avoid the use of such substances except where strictly necessary and under rigorous control.

Other potential risks may lie in incorporation of transgenic DNA into the genomes of resident gut microflora (though this is likely to be very improbable) or a change in the pathogen spectrum of the transgenic fish leading to it hosting a new pathogen which happens to be also a human pathogen.

Maclean and Laight (2000) assessed risks to consumers as "very low".

9.2 Biodiversity

The extent of aquatic diversity is both extremely large and relatively poorly understood (Beardmore, Mair and Lewis, 1997). This means that the task of estimating the risks to aquatic biodiversity at all of its levels from the use of GMOs or indeed, any genetically distinctive strain used in aquaculture is monumentally large. Aquaculture has a further problem in that the (almost always unintended) escapes of genetically distinct farmed fish are unpredictable and often large in numbers. Stenquist (1996) in discussing transgenics in open ocean aquaculture, quotes some relevant figures. Thus, 15 percent escapes for Atlantic salmon, escapes of 150 000 salmon and 50 000 trout in Chile and catch statistics for Atlantic salmon off Norway in which 15−20 percent of the fish caught were of farmed origin. In Scotland an escape of 100 000 Atlantic salmon was reported recently. It is clear that escapes of these magnitudes pose considerable problems and it is not surprising that in some parts of Norway fish of farmed origin represent a majority of the animals fished (Saegrov *et al*., 1997)

The major focus of attention in the literature lies, understandably, upon the effects of escapes upon natural populations of the same species, but we must always bear in mind possible impacts across an assemblage or ecosystem as a whole. The first general point to make is that there is, in principle, no difference between the biodiversity risks from escapes of GMOs and from fish genetically improved in some other way, e.g. by selective breeding or (in some respects) from exotic species.

The second general principle is that such genetically improved forms including GMOs, are developed for a specific set of environmental circumstances in which they enjoy an advantage conferred by human decisions. In nature, however, such genetically distinct forms may legitimately be regarded as mutant forms of the wild type. A considerable body of genetical knowledge tells us that the probability of survival of mutant forms is extremely low because they are disadvantaged in viability and/or fertility under natural conditions. Thus, for example, in the genetically distinct farmed Atlantic salmon in Norway the males are very much less successful than wild males in securing mates (Jonssen, 1997).

However, it must be conceded that in species like salmon where the farmed populations outnumber the wild populations by orders of magnitude, the effects of escapes of any genetically distinct genotype upon natural populations may be both deleterious and of significant size simply as a result of "swamping"

An interesting model of the effects on a medaka (*Oryzias latipes*) population of transgenic release has been produced by Muir and Howard (2001) using estimates of juvenile and adult

viability, age at sexual maturity, female fecundity, male fertility and mating advantage. They were able to demonstrate that the transgene would spread in natural populations, despite low juvenile viability, if transgenes have sufficient high positive effects on other fitness components. It has been argued that this might lead to extinction but the selective pressure for recombinant genomes with higher viability would be expected to be immense.

Maclean and Laight (2000) simulated the changes in frequency of a transgene expected with different scenarios embracing a range of selective values including heterozyote advantage. They note that "repeated small introductions [of the transgene] can have an effect on … frequency … since the frequency of advantageous alleles rises much more rapidly than if a single large introduction is considered".

A major problem in assessing risk to natural populations is that of scale. Even if farmed fish are at a selective disadvantage in natural conditions, the ratio of wild:farmed numbers may in some areas, be relatively small. In these situations significant modification of the "native" population and its role in the ecosystem is inevitable.

Whilst not providing a completely satisfactory answer, there is little doubt that making farmed fish sterile would go a long way towards reducing the pressure upon such threatened ecosystems. A number of research efforts to develop systems for sterile fish production are being made. The techniques include triploidisation, antisense transgenics, ribozymes and gene targeting (Maclean, 2002; Uzbekova *et al*., 2001; Maclean, pers. com.).

Provided that the best containment measures (physical and biological) are adopted, in our opinion, in general risks to biodiversity by GMOs *per se* are probably extremely small, but in specific cases, the risks and consequences may be large. As a general rule and adopting a precautionary approvah (OECD, 1995), it is, however, clear that each individual case needs careful study and appraisal and the best possible containment measures before approval for uptake into commercial production is given.

9.3 Animal welfare

The direct or indirect effects of transgenesis upon the welfare of fish GMOs in aquaculture are very poorly understood. In part, no doubt, this is because notions of cruel or unnatural treatment in mammalian species translate, for a variety of reasons, imperfectly to fish. Nevertheless, as life forms with highly developed nervous systems and with a range of behavioural phenotypes which flow from this, fish qualify for welfare consideration.

There are a few studies which bear on this. Thus, for example, Devlin *et al.* (1995b) reported changes in colouration, cranial deformities and opercular overgrowth and lower jaw deformation in coho salmon transgenic for AFP and GH. After one year of development anatomical changes due to growth of cartilage in the cranial and opercular regions were more severe and reduced viability was evident.

The larger body of data on species farmed terrestrially shows dysfunctional development leading to acromegaly, lameness and infertility in some GH transgenics in pigs and sheep. However, in pigs dietary modification influencing nutritional levels of zinc proved successful in avoiding such abnormalities (Pursel and Solomon, 1993; Pursel, 1998).

We have been unable to find systematic data on the incidence, in fish GMOs, of effects such as those described by Devlin *et al*. (1995b) and this is probably because animal welfare is not

sufficiently widely recognised as an issue in relation to the use of GMOs. This is well illustrated in the otherwise comprehensive and balanced review by Sin (1997) in which the section on ethical issues contains no reference to animal welfare. Nevertheless, if GMOs are to be used in aquaculture (and there are weighty arguments for so doing), concerns on this issue will need to be properly satisfied. The Royal Society report (2001) devotes a significant amount of space to this issue.

9.4 Poor communities

This term rather than poor countries is used because all poor countries contain rich people and rich communities. The possible economic disadvantages of use of transgenics centre on two issues:

9.4.1 Dependence on external agencies for seed fish

If transgenic fish become widely grown because they are much more efficient, and if special broodstock are required to produce fry for on-growing to adults, which, cannot be used as broodstock, a dependency is created. This dependency may be benign or oppressive, depending on the arrangements made for seed supply.

9.4.2 Intellectual property rights

This is a very difficult issue indeed. Since genes may now be patented and therefore, enjoy commercial value, the opportunities for dispute about equitable treatment of stakeholders in cases where ownership of genes and strains is contested, are legion.

A recently published report (Commission on Intellectual Property Rights, 2002) states that developing countries are frequently disadvantaged in the use of, and access to, IPR because of increasingly protective attitudes taken by owners of IPR. However, the report also indicates that developing countries are very heterogeneous in respect of their ability to use and develop IPR.

10. REGULATION, POLICY AND THE CLIMATE OF ACCEPTANCE

The extent to which GMOs are perceived as desirable for aquaculture and food supply has been probed by Bartley and Hallerman (1995) on a questionnaire basis. The responses were generally positive in terms of exploring the potential of transgenesis. It is clear however, that in human populations as a whole there is a severe deficiency of knowledge appropriate for making informed decisions about the value of GMOs (Dunham, 1999).

The task of instituting and managing well thought out, responsible and scientifically sound measures is made more difficult by the frequently irresponsible and inaccurate media treatment of GMOs on the one hand and the cavalier pronouncements by some authorities on the other hand, e.g. that no distinction need by made in labelling between food derived from GMOs and from non-GMOs. Such extreme differences in attitude tend to inflame public opinion.

The need for incorporation of risk assessment and risk management procedures relating to use of GMOs in aquatic species has been well brought out by Hallerman and Kapuscinski (1995).

According to Hallerman and Kapuscinski (1995), "as a generality among developed countries at least, the public will support biotechnology if it yields a healthful product in an environmentally sound manner. This statement encapsulates implicitly a most significant factor – that in considering the benefits and disadvantages of GMOs**,** perception by the general public is a most important factor in shaping attitude of regulatory agencies" (emphasis by JAB and JP).

There is at least a suspicion that some contemporary science may also be affected by perceptions. A recent furore over the alleged presence, in traditional varieties of maize in Mexico, of transgenes derived from modern strains of cultivated maize points to this. The matter is still not completely resolved but has led to *Nature* declaring it should not have published the original paper because of technical inadequacies (Mann, 2002).

It is perhaps dangerous to extrapolate directly from agronomy to aquaculture but it is worth noting that, in the USA, which accounts for more than two thirds of the acreage planted to crop GMOs, moves to institute a much tighter regulatory framework are in train (Soil Association 2002). Five bills were introduced in May 2002 in the House of Representatives in Washington to cover legal protection for farmers, increase GM food safety, require labelling for foods containing, or produced with, GMOs, address developing country issues and assign liability for damages. These measures appear to have sprung, at least in part, from serious dissatisfaction on the part of some farmers with GM crop plants. It is, however, relevant to note that the dominant position enjoyed by several large multinational agrochemical companies in providing both seed and agrochemicals is not reflected in the aquaculture industry which is far more fragmented. Nevertheless, the indications are already present that there is a move towards fewer and larger companies within the sector and this could have consequences for the control and use of GMOs in aquaculture species.

A fair number of countries have instituted regulatory arrangements for the culture, release and dietary utilisation of GMOs, but with considerable differences in the approaches and restrictions employed (discussed by Maclean, 1998).

At the international level the Cartagena Biosafety Protocol of the Convention on Biological Diversity provides a comprehensive and rigorous framework for regulation of protection of biodiversity and argues that this be done **"**taking into account risks to human health and specifically focussing on transboundary movements". The great majority of nations are signatories to the convention. What is less clear is the extent to which, at national and regional levels, the protocols set out are implemented and implementable through domestic legislation and effective sanctions.

Among its provisions, the Cartagena protocol has a detailed treatment of risk assessment in relation to the protection of biological diversity. Among the important and relevant general principles are:

1. "Risk assessment should be carried out on a case by case basis."

2. "Risks associated with LMOs should be considered in the context of the risks posed by the non-modified organisms in the receiving environment."

and in the [comparable] methodology of risk assessment:

1. "An estimation of the overall risk posed by the LMO based on an evaluation of the likelihood and consequences of the adverse risks being realized."

2. "A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks."

We are, thus, faced with a complex array of stakeholders; breeders and growers, geneticists, multinational companies, supermarkets, consumers, politicians and the media. All have their own agenda to follow, but critically important to all of them and to a reasonable resolution of the current conflict is the availability and use of full, sound and accurate information, particularly in the context of framing of appropriate regulations affecting GMOs

11. CONCLUSIONS

- GMOs in aquaculture have much to offer in terms of improvements in aquacultural production, food security and generating economic benefits.

- GMOs will undoubtedly be used in aquaculture but use should be in conformity with principles of the Cartagena Protocol.

- Greater precision and efficiency in the techniques of induction of transgenics will need to be developed, particularly with respect to sites of integration.

- Integrated sequences should not contain DNA of viral origin, reporter genes or other genes not required for the target phenotype.

- The risks attached to the use of GMOs need to be analysed and quantified in more realistic and reliable ways than so far is the case.

- The use of (reversibly) sterile fish for production offers a route for reducing, very considerably, risks to biodiversity from the use of GMOs.

- There is an urgent need for balanced and accurate information on GMOs to be disseminated among policymakers, aquaculturists and the general public.

- Regulatory frameworks for the exploitation of GMOs are necessary but should be based upon reliable, objective criteria.

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