

Utilization of modern biotechnology for development of aquaculture: a review.

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ABSTRACT:

Biotechnology is now an established strategy for induction of transgenesis in fish and many different fish species have been modified in this way. These species include many commercial food fish such as tilapias, catfishes, rainbow trout, salmonids, arctic charr, carps, northern pike, and other fish species which serve as model experimental systems especially in developmental genetics like goldfish, medaka and zebrafish. The properties modified or attempted for modification are growth enhancement, improvement of disease resistance and cold tolerance and freeze resistance, altered glucose metabolism, sterility and the exploitation of fish for the production of pharmaceuticals .

Since fish are highly mobile animals, the release or escape of cultured transgenic fish to natural waters could transfer modified genes into wild stocks. We discuss the major risks and the potential benefits of using biotechnology in aquaculture .

Containment of transgenic fish may be achieved by limiting culture to safely enclosed systems, or by the imposition of complete sterility on the transgenic fish. Such sterility could in some cases result

from triploid induction: in other cases, it could probably be achieved through gene manipulation .

1. Introduction

The ever increasing world population imposing serious challenges for food supplies. Fish accounts for nearly 20% of consumed animal protein and approximately one billion people across the world rely on fish as a primary source of animal protein. Total fisheries production (including molluscs, crustaceans and fish) exceeded 99 million metric tonnes in 2003, while agriculture contributed a further 35 million metric tonnes (FAO, 2005).

The beneficial health properties fish consumption due to the omega 3 polyunsaturated fatty acids(ω 3-PUFA) found in many marine fish such as tuna, mackerel and salmonids will be an additional motivation for elevating the demand for fish as food.

Capture fisheries are declined as a result of over-fishing (Hutchings, 2000); so aquaculture is expected to contribution more in the world protein supplies. However, aquaculture faces criticism as a source of environmental pollution and ecological degradation, suggesting that its rapid growth will come with some problems.

It is commonplace to think of aquaculture in the light of technologies, which allow intensification of the culture, partly because of strict physical limits to the wide extension of aquaculture to new geographical regions. One possible solution would be using biotechnology to produce new and better strains of fish for aquaculture. However, such a development could face various and difficult problems. In this review, the

potential of the biotechnology as applied to aquatic organisms, especially fish is considered, and the benefits and potential hazards of such development evaluated.

2. Historical background and definitions

After innovation of molecular biology techniques, cloned genes became available in the early 1970s to modify genetic material by introducing novel genes to an organism on a single gene basis. An early breakthrough allowed to transform mouse embryos following injection of purified DNA, and mice with enhanced growth were produced following egg injection with a growth promoting gene construct (Palmiter et al., 1982). This raised the prospect of applying gene manipulation to fish. Certain complications were apparent, in that the fertilized fish egg contains tiny pronuclei which deny microinjection. Thus while mammalian eggs allow nuclear injection, extension of the technology to fish required placement of the cloned gene copies in the fish egg cytoplasm in the hope that some of the copies would find their way into the nucleus, thus allowing chromosomal integration and consequent genetic transformation of the organisms (Maclean et al., 1987).

Confusing terminology has arisen in line with the transgenic revolution. GM is taken to mean "genetically manipulated", a GMO is a "genetically modified organism" and LMO is a "living modified organism". These acronyms tend to be used interchangeably, all referring to transgenic organisms, and fall short of the ideal in that technically, triploid fish are genetically manipulated, but are commonly excluded from the GM umbrella. Thus, GM is perceived to include only organisms that are genetically modified by the "GM technology", namely artificial

insertion of so-called novel genes. Nevertheless, since genes from the same organism can rarely be deemed novel, even this definition is still problematic.

3. Overview on gene manipulation

A range of methods has been used to introduce cloned genes into fish. Microinjection into the fertilized egg is the most widespread approach, but electroporation, liposome-mediated gene transfer, and gene guns have all been tried, and some authors favored electroporation (Hostetler et al. 2003), especially with medaka (*Oryzias latipes*). Eggs of salmonids and tilapias are rather difficult to microinject because of the tough chorions and the opaque nature of the eggs. On the other hand, species such as common carp (*Cyprinus carpio*), zebrafish (*Danio rerio*) and catfish (*Ictalurus punctatus*) produce transparent eggs with soft chorions, that can be injected even with hand-held needles. Microinjection of unfertilized fish oocytes has also been successfully achieved in medaka by Ozato et al. (1986).

To take Nile tilapia (*Oreochromis niloticus*) as an example of a fish widely used in genetic modification experiments, the methodology is roughly as follows. Transgene copies, which consist of the selected promoter sequence, spliced to a coding gene (either cDNA or genomic) and a 3 prime polyadenylation sequence, are produced in bacterial plasmids and recovered by restriction enzyme digestion, so that only linear copies of the transgene construct are injected into the eggs. The DNA is dissolved in NaCl Tris- buffer at a concentration such that the injection volume of approximately 250 picolitres per egg will carry 2×10^5 copies of the transgene. Eggs are fertilized in vitro after stripping of

reproductive male and female fish, and injected within one or two hour's fertilization. Microneedles used for injection are drawn to an end with an internal diameter of approximately 5 microns. The injection needle is linked to a specially designed picoinjector so that injection volume and pressure can be precisely controlled (Rahman and Maclean, 1992; Maclean et al., 2002; Maclean, 2003).

In the case of tilapia, a well trained operator will be able to inject about 50 - 70 eggs within the window of the one cell stage, which lasts at most two hours after fertilization. Following injection, eggs are incubated and the hatched fry reared for subsequent assay. Batches of fry may be pooled for polymerase chain reaction (PCR) assay, but in order to determine the incidence of transgene integration into the genome, it is best to wait until young fish became approximately six weeks old, by which time fin clips can be used to test for integrated copies by PCR. Since the transgene copies often combine end to end in solution, it is common to find that the integrated sequence is in fact a concatemer of multiple copies of the original construct(Fig.1)

From 100 fertilized eggs injected, a common outcome is that approximately 80 will survive to the fry stage, of which 50 may exhibit some transgene expression. However, this early expression is mainly transient expression of unintegrated copies and is not indicative of true integration. When six weeks old fries are monitored, all unintegrated transgene copies will be disappeared (except in rare cases of nuclear persistence of unintegrated copies, as has been recorded in carp, *Cyprinus carpio*, by Zhang et al., 1990), and expression assays and PCR for DNA transgenes are diagnostic of true integration. The number of positive fish

at this time is likely to be between 1 to 10 out of the original 100 eggs injected. Such positive specimens are consistently mosaic with respect to the cellular distribution and expression of the transgene copies, due to the delayed integration of transgene copies until the embryo reaches the 4 cells stage or more. This also causes problems of germ line transmission of transgenes from these original transgenic fish, which is less than the incidence of mosaic positives, and is likely to involve between 0.1 and 1.0% of the original 100 eggs and their embryos.

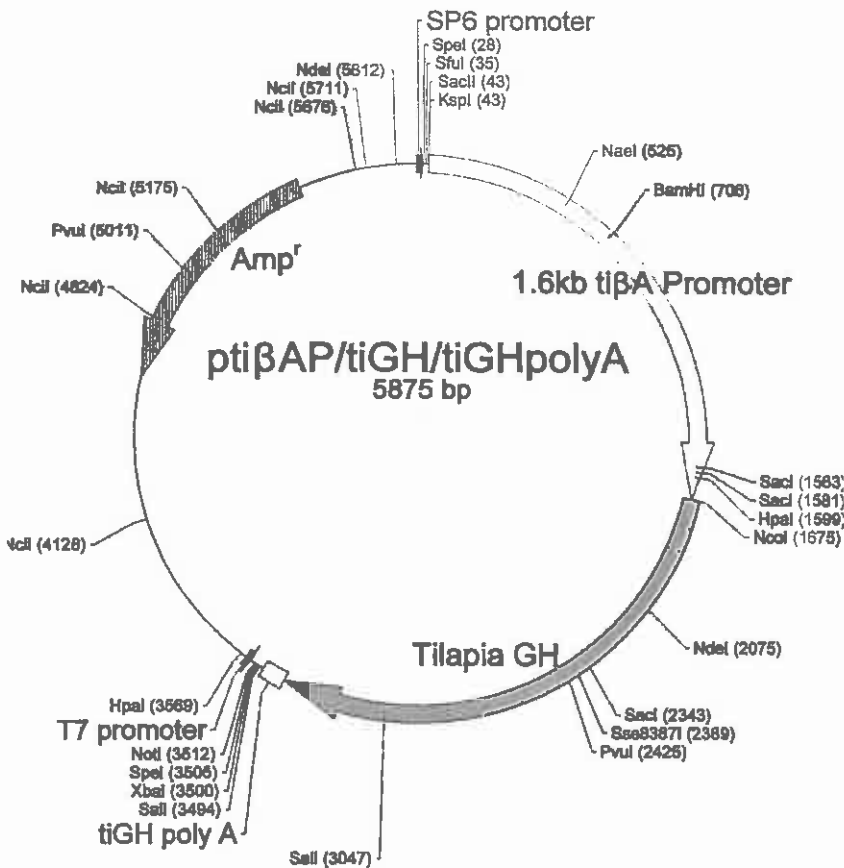


Fig. 1. Diagrammatic representation of the 'all tilapia' growth hormone gene construct currently in use. The diagram shows the entire plasmid. The linear sequence used to generate the transgenic fish includes only the

tilapia beta-actin promoter (1.6 tibAP) and the tilapia growth hormone coding sequence plus the relevant polyadenylation sequence (tiGH). All these sequences are from a genomic library of *Oreochromis niloticus* (Maclean, 2003).

Because of mosaicism phenomenon, these germ line transmitters may also transmit to low numbers of G1 progeny, even less than 1 % in a cross with a wild type fish. However, if multiple chromosomal integration events have occurred in a particular transgenic fish, then more than 50% G1 positives may be recovered. All G1 positives are hemizygous, in that a transgene is only incorporated into one of a pair of homologous chromosomes. However, by crossing two G1 positive fish in the same line of fish, G2 progeny will consist of 25% homozygous transgenics, 50% hemizygous transgenics, and 25% homozygous wild type (Anderson and Pauley, 1992; Collas and Alestrom, 1998; Rahman et al., 2000; Hostetler et al., 2003).

Some further aspects of this biotechnology and variations of the particular approach can be usefully listed as follows:

4. Fish species and lines produced

Fish species which have been subjected to biotechnology fall into two groups. The first is species that offer no commercial potential but are good model species for experiments on gene regulation and developmental biology, as well as proving useful for the preliminary testing of gene constructs that will subsequently be used in other fish species of commercial significance. This group consists of zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), and goldfish (*Carassius*

auratus). The second group is species that may be used as model experimental systems but are also of potential commercial significance such as Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), channel catfish (*Ictalurus punctatus*), common carp (*Cyprinus carpio*), Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), Arctic charr (*Salvelinus alpinus*), rainbow trout (*Oncorhynchus mykiss*), northern pike (*Esox lucius*), loach (*Misgurnus fossilis*), and mud loach (*Misgurnus mizolepis*).

5. Present and future research

There are a number of more commercially orientated objectives along with the use of transgenic fish to explore problems in genetics and developmental cell biology. These include growth enhancement, improvement of disease resistance, cold tolerance and freeze resistance, more efficient metabolism, sterility and the use of fish as biofactories.

5.1. Growth enhancement

The mice produced by Palmiter et al. (1982), and the experiments that followed this approach, were larger than their non-transgenic littermates. This was because the promoter sequence used to drive the growth hormone (GH) coding gene in the construct resulted in GH expression from the liver rather than a small part of the pituitary tissue. This outcome accomplished by using a liver-specific promoter associated with a gene for metallothionein. In a similar way, fish of various species have been successfully growth-enhanced by combining a GH coding sequence with a promoter that is widely expressed such as those for metallothionein (a metal binding protein), beta actin (a ubiquitously

expressed cytoskeletal protein) or antifreeze (a protein made in the livers of Arctic fish and secreted later into the blood).

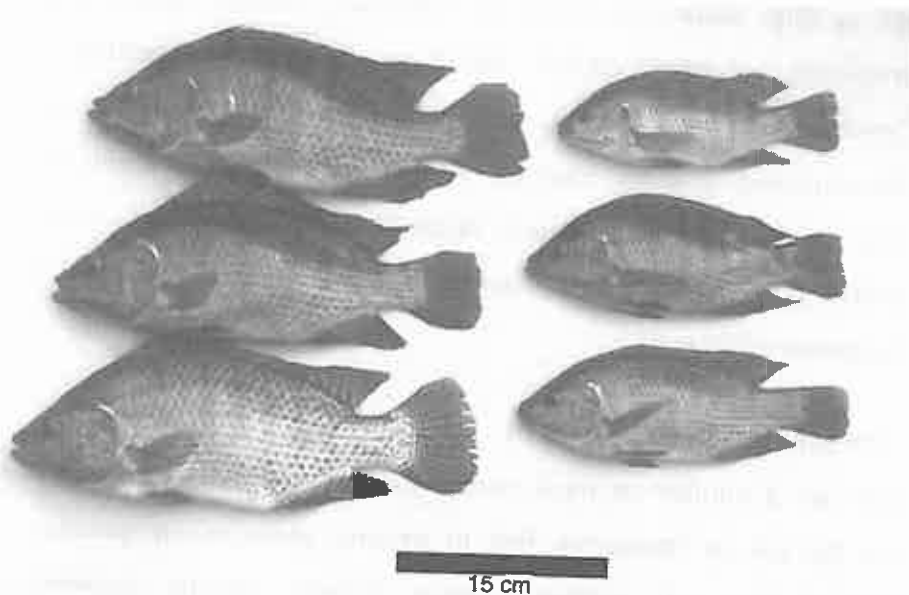


Fig. 2. Comparison of growth enhancement and control tilapia from the trials of Rahman et al. (2001). Fish are 30 weeks of age, the three on the left being transgenic and those on the right controls. The average weight of the transgenic and non-transgenic in this group was 653 and 260 g respectively and the fish illustrated were representative of the entire batch.

Fish growth is very flexible, and more or less continuous through life. It is also the case that few fish species have been subjected to long-term selection for growth as have other food animals. For these reasons, some fish show dramatic growth responses of more than ten fold when made transgenic for GH constructs (Du et al., 1992; Devlin et al., 1995; Nam et al., 2001). Since dramatically increased growth may produce skeletal abnormality, it is desirable to aim only doubling or trebling of growth. Increased growth of Nile tilapia (*Oreochromis niloticus*) of two or

three fold at seven months has been achieved (Rahman et al., 1998 and 2001) (Fig.2). There is evidence that some species such as common carp show less dramatic growth increases, because they have already been selected for optimal growth over centuries (Zhang et al., 1990). Since the dramatic increase in size of fish is not commercially desirable and may lead to abnormality, it is beneficial to select transgenic strains that have only single novel GH genes added to the genome and only show modest increases in GH levels(Ostenfeld et al. ,1998). Since promoters from many different fish species are now available, there has been a recent trend to use constructs of entirely fish origin, and to use constructs entirely derived from the same species. This so-called 'autotransgenic' approach has been followed successfully with many species (Chourrout et al., 1986; Dunham et al., 1987;Gross et al., 1992;Krasnov et al., 1999; Nam et al. ,2001) .There is no evidence to date that growth-enhanced transgenic fish have been used commercially, but some have been exposed to limited field trials in a contained situation (Rahman et al., 2001).

5.2. Improving disease resistance

It is difficult to design transgenic fish with improved disease resistance, because genes conferring this property are hard to identify. This is unfortunate because aquaculture involves culture at high densities so diseases spread easily and is hard to control. Indeed, this is one property to which biotechnology could usefully contribute in aquaculture. There are two examples of experiments to develop fish with increased disease resistance. One is the work of Hew et al. (1999) on producing transgenic Atlantic salmon with rainbow trout lysozyme cDNA, driven by

an ocean pout antifreeze promoter. This lysozyme has already been demonstrated to have antimicrobial properties against a range of Gram-negative bacteria such as *Vibrio* and *Yersinia* sp. (Grinde, 1989) which are also fish pathogens. Unfortunately no positive outcome has been reported from this work. A more promising outcome has emerged from the work of Dunham et al. (2002) in which channel catfish made transgenic for cecropin genes which are natural anti- microbial proteins found in insects. An increased level of disease resistance has been reported for these fish

5.3. Improving cold tolerance or freeze resistance

There are two separate traits, cold tolerance being the ability to thrive at temperatures above 0°C but below the normal physiological limits for that species, while freeze resistance is the ability to stop ice formation in tissues in water at less than 0°C. The phenomenon of cold tolerance is important in carp culture in north China, where severe losses occur with common carp. Similarly, large numbers of tilapia have died in Palestine when winter water temperature dropped below 10°C. Freeze resistance is a common and remarkable property of many Arctic and Antarctic fishes, which secrete antifreeze proteins into blood and other tissues to prevent the formation of ice crystals.. The commercial interest in producing transgenic fish which are resistant to freezing comes from the desire to culture Atlantic salmon in sea cages in northern Canada. Severe losses can occur in some winters when icebergs float southwards. The evidence that fish can be made more cold tolerant by biotechnology rests on the observation that transgenic goldfish with an antifreeze gene construct were protected from the deleterious effects when cultured at low temperatures (Wu et al., 1998). One explanation for this was that

antifreeze synthesis may reduce membrane permeability and so afford some physiological benefit.

Strains of transgenic Atlantic salmon have been produced which express antifreeze from integrated copies of an antifreeze gene from the winter flounder (*Pseudopleuronectes americanus*) (Fletcher et al. ,1992; Hew et al. ,1999). Although integration, expression and transmission of the antifreeze genes were achieved, the level of antifreeze protein produced remains low and no serious freeze resistance was evident .

5.4. Modified metabolism

This factor is a good example of potential improvement of cultured fish which has been neglected in attempting biotechnology. Salmonids, now cultured intensively in many countries, are essentially carnivorous fish. They also have poor abilities to utilize carbohydrate, despite the presence of the normal insulin active pathway of carbohydrate metabolism (Wilson, 1994). A research group tried to alter the metabolism of Arctic charr (*Salvelinus alpinus*) with respect to hexokinase and glucose transporter genes, using biotechnology (Krasnov et al., 1999). Altering salmonids metabolism will not be easy, but the results could be of great importance.

5.5. Sterility

Since sterile fish play a basic role in the commercial exploitation of transgenic fish, this is an important factor to investigate. Non-transgenic sterility can be accomplished by the induction of triploidy. Sterility via genetic modification will be a useful genetic development if it was reversible, so that brood stock can be retained for production of

further sterile progeny. Since there is no immediate prospect of gene 'knock-out' in fish via the exploitation of embryonic stem cell lines to accomplish this end, it is necessary to attempt other approaches for sterility via gene 'knock-down' as in tilapia (Zhao et al., 2001; Maclean et al., 2002). Some success with this methodology reported in rainbow trout (Uzbekova et al., 2000), and potential success in tilapia (Maclean et al., 2002).

5.6. Production of pharmaceuticals

For many reasons fish are good model systems in which valuable human proteins may be transgenically expressed. Some of these reasons are that fish are generally cheap, easy to culture intensively, often have short generation times and have more or less continuous reproductive activity. In addition, work on fish is more ethically acceptable than using mammalian or avian models, and there is no present evidence for the replication or transfer of prions in and from fish. There are a number of possible scenarios by which interesting and valuable proteins can be synthesized in and recovered from fish. One is to make such proteins in fish eggs and recover from the eggs, another is to organize synthesis in the embryo and recover from embryos, and the third is to develop lines of transgenic fish in which a particular organ or tissue is the site of synthesis and thus the final step is the recovery of the protein from the specific organ. Two procedures have been used with fish for production of human factor VII, a blood clotting factor that is activated following internal tissue injury. In the first, human factor VII was expressed in and recovered from tilapia embryos, following egg injection with transgene copies. Although,

small amounts of protein were recovered in the preliminary experiments, the system seems promise (Hwang et al., 2004).

In the second procedure, lines of transgenic tilapia have been produced in which human factor VII is synthesized in the liver. The transgene used consists of a cDNA for human factor VII driven by a tilapia vitellogenin promoter. Factor VII is secreted into the blood, from which it is readily purified. Although substantial production levels have not achieved and the folding and glycosylation of the resulting protein verified, it has already been shown to efficiently clot human cells.

6. Commercial Exploitation

Up to the present time there are no examples known of the commercial exploitation of transgenic fish, except for strains of zebrafish sold through the pet trade which are transgenic with respect to the reporter gene red fluorescent protein the gene of which has been recovered from jellyfish species. These fish, marketed as Glofish in the USA. Surprisingly the FDA found no reason to regulate these fish and so they are freely available to the pet trade. A number of experts stated that the fish do not pose a potential environmental threat since they appear to be less fit than wild-type zebrafish and so, if they escaped and interbred in the wild, progeny would not survive for long.

Transgenic Atlantic salmon are the nearest to commercial use and has applied to the US FDA for permission to develop and market.. The application is for the use of all-female triploid fish -, thus giving a very high degree of biological containment through sterility. Semi-field trials of various transgenic fish have been undertaken, notably with channel

catfish in USA (Dunham et al., 1992) and tilapia in Hungary (Rahman et al., 2001).

7. Advantages and disadvantages of transgenic fish

7.1. Advantages

The main advantage of biotechnology is allowing modification, enhancement or negation of genetic traits, on a gene by gene basis. Conversely, traditional breeding, suffers from the loss of other genetic traits or uncovering accidentally during the selection, so that the new breed has one distinct advantage over the original brood stock but also may have new disadvantages.

A particular aspect of the ability to add or delete one gene at a time is also that the novel gene can be teamed with a different promoter, so ensuring an altered pattern of gene expression, and that the gene can be isolated from quite unrelated species, so that the ability to make say, antifreeze protein is bestowed on Atlantic salmon from winter flounder. All of the properties discussed in the earlier parts of this review have the potential to be substantial advantages.

7.2. Disadvantages

A) There is the possibility that the incorporation of a novel gene on a random basis within chromosomal DNA could lead to unforeseen genetic defects due to insertional mutagenesis. Also, position effects may result from incorporation, such that genes adjacent to the incorporated novel gene now have changes in their regulation or expression levels. However, this is infrequent in fish (although, if lethal, it can be easily missed in a

low % embryonic survival), due to the large amount of non-functional DNA in the genome.

B) Transgenic fish can often incorporate multiple concatenated copies of transgenes, leading to overexpression of the trait of interest. This is especially evident in some growth-enhanced lines overexpressing growth hormone.

C) There is a theoretical possibility that incorporated transgene copies could be subsequently lost, or silenced by DNA methylation. There are examples of non-expressed transgenes in some lines of fish, but not of gene elimination or silencing of a previously active transgene.

D) Transgenic fish may have the capacity to become a pest species on escape or introduction.

However, there are many genetic considerations which should be taken in evaluating advantages and disadvantages of this modern technology (Devlin et al., 1995; Jonsson et al., 1996; Martinez et al., 1996; Muir and Howard 1999; Hedrick 2001; Nam et al., 2001)

8. Risks to aquatic environment

As outlined earlier briefly, there are number of alternative scenarios to consider, depending on whether the transgenic fish are sterile or reproductive, on whether non-transgenic conspecifics are present in surrounding water, and of course depending on the level and type of containment employed (Muir and Howard ,1999 and 2001).

8.1. Effective containment

Enclosed water systems offer one form of effective physical containment, provided that there are rigorous measures to control escapes to the wild, or accidental movement of eggs or small fish by bird predation, or theft. Countries such as Canada and Finland have numerous landlocked lakes which offer opportunities for contained culture of salmonid fish while countries such as Iceland and Hungary have warm geothermal water sources surrounded, especially in winter, by cold water which would not allow survival of species such as tilapia (Johnsson and Bjornsson, 2001).

This type of containment was exploited by Rahman et al. (2001) for the growth testing of transgenic tilapia (Fig.2).

Effective containment can be achieved also by construction of elaborate barrier systems. This has been implemented for the experimental culture of both carp and channel catfish in the United States (Donaldson, 1997). Sometimes the effective biological sterility of an introduced species allows containment, as in the introduction and release of rainbow trout in European waters, in which breeding records are rare. European aquatic environment may lack some cues that are essential for reproduction in this species (Maitland and Campbell, 1992; Farrell et al., 1997).

Biological containment via induced sterility becomes especially significant with transgenic fish via triploid induction (Benfey, 1999). Triploidy is normally induced by heat shocking or pressure shocking of the fertilized eggs and these methods have been widely applied to salmonids (Benfey and Sutterlin, 1984). The efficiency of triploid induction varies greatly with species and is often less than 100%. In addition, the triploid fish may be partially fertile, especially in the case of

male fish. To optimize sterility, triploid induction can be combined with sex reversal to produce all-female progeny, and this is the scenario used in the production of the transgenically growth enhanced Atlantic salmon.. However, in fish such as tilapia this procedure is not attractive since the male tilapia is much the more marketable fish(Donaldson ,1997; Maclean and Laight , 2000).

Transgenesis itself can be implemented to produce sterile fish, as reviewed by Maclean et al. (2002) in the case of tilapia. This approach involves blocking expression of a hormone which is essential for gonadal development, such as gonadotropin (GtH) or gonadotropin releasing hormone (GnRH), and rescuing brood stock fish by intra-muscular injection of the relevant reproductive hormone. In the absence of gene 'knock-out' methods in fish, it has been necessary to rely instead on targeting of the specific messenger RNA, so called gene 'knock-down'. Although double stranded RNA (RNAi) and mRNA targeting via ribozymes have to date proved ineffective in fish, some positive results have been reported for the use of antisense strategy against specific mRNAs (Uzbekova ,2000; Maclean et al. ,2002).

If sterility is watertight, it would allow the use of transgenic fish even when conspecifics were present, which is always the most risky situation. If conspecifics are not present in the environment, sterility would still be attractive to prevent transgenic fish establishing as an exotic introduction.[For more details about different situations and risks see Kapuscinski and Hallerman (1991), Maclean and Laight (2000), Muir and Howard (2001) and Knibb (2002)] .

8.2. Food safety

All transgenic modifications to fish involve genes which code for proteins, and clearly if transgenic fish are eaten, their proteins, including the products of the transgenes, will be digested in the usual way. The proteins produced to date in transgenic fish include GH, lysozyme, cecropin, antifreeze and reporter gene products such as lacZ and green fluorescent protein. The most recent lines of transgenic fish that have been developed are transgenic only with respect to sequences from the genome of the same fish, so no novel proteins are involved. Important considerations with respect to the development of transgenic fish which are destined for the food market are :

[i] If genes coding for antibiotics or antibiotic resistance were included in transgene constructs, such protein could harm the intestinal flora of the consumer. The antibiotic resistance gene *neo* (a gene of bacterial origin which confers neomycin resistance and is commonly tested with the neomycin analogue G418), has only been used within the last decade with model species such as medaka and zebrafish.

[ii] Some fish genomes harbor genes which code for toxic proteins. If any of these proteins are coded in the fish genome (some, such as those of the widely eaten but potentially lethal Japanese puffer fish, are acquired by the fish secondarily), then clearly such genes must never be implicated in the biotechnology.

[iii] Genes or regulatory sequences of viral origin should be avoided, although viral promoters have been employed in fish transgenes in the past.

[iv] There is a theoretical possibility that transgene-derived proteins could be immunogenic to some people, in much the same way as some

individuals are hyper-sensitive to nut proteins or Crustacean proteins. However, none of the proteins involved in transgenic fish production are known to present such problems.

[v] Although there are no known reasons why transgenic fish should prove harmful or have an altered appeal as food, some have been tested in this regard in order to provide public reassurance. This has involved transgenic tilapia (Guillen et al., 1999) and trout (Entis, 1998).

9. Concluding remarks

During the course of development, scientists and have learnt many about responding to naive questions from the public about the risks. But quantifying risk, especially in the context of transgenic fish, is extremely difficult. Culture of non- transgenic fish carries numerous risks, as does the sale and consumption of the resulting food product. It is undeniable that transgenic fish add to these risks. If commercialization of transgenic fish occurs, each transgenic line will have to be separately authorized. Necessary information prior to authorization will have to include full details of the transgene sequence used, any known genetic position effects or physiological changes in the fish, and precise details about the novel proteins now present than are not present in equivalent non- transgenic strains.

In conclusion , production of transgenic fish by biotechnology my prove useful in countries that lack permanent open inland waters where transgenic can escape , so effective containment is in effect , as in Libya and many other North African and Sahara countries. However, this should be considered carefully to avoid any unexpected and unwanted outcomes of such practice.

استخدام التقنية الحيوية الحديثة في تطوير الزراعات المائية: دراسة مرجعية

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المستخلص

تعتبر التقنية الحيوية الآن من الخطط الأساسية لتحفيز التحول الوراثي في الأسماك وقد تم تعديل العديد من أنواع الأسماك بهذه الطريقة. وتضم هذه الأنواع العديد من أسماك الغذاء التجارية مثل البلطي وأسماك القط والتراوت القرحي والسالمونيات والشار القطبي وأسماك الكارب والبايك الشمالي، وأنواع أخرى تعمل كأنظمة تجريبية خاصة في دراسات الوراثة التطورية مثل السمك الذهبي والميداكا والأسماك المخططة. والخصائص التي عدلت أو استهدفت بالتعديل هي تعزيز النمو وتحسين مقاومة الأمراض وتحمل البرد ومقاومة الانجماد وتغيير أيض الجلوكوز والعقم واستثمار الأسماك لإنتاج المركبات الدوائية.

ولأن الأسماك حيوانات كثيرة الحركة فإن إطلاق أو هروب الأسماك المعدلة وراثيا المستزرعة إلى المياه الطبيعية يمكن أن ينقل معه الجينات المعدلة إلى داخل التجمعات الطبيعية. وقد أوضحنا المخاطر الرئيسية والمزايا المحتملة لاستخدام التقنية الحيوية في الزراعات المائية.

وربما يمكن تحقيق احتواء الأسماك عن طريق تقييد التربية بالأنظمة المغلقة بأمان أو بفرض العقم التام على الأسماك المعدلة وراثيا. وهذا العقم يمكن أن ينتج في بعض الحالات من تحفيز تعدد الصبغيات وفي حالات أخرى ربما يمكن تحقيقه من خلال تعديل المورثات.

9. REFERENCES

1. Anderson, W.I. and Pauley, B. (1992) **Avoidance and attack behaviors of transgenic Atlantic salmon. J. Fish Biol., 50:218-232.**
 2. Benfey, T.J. (1999) **The physiology and behavior of triploid fishes. Rev. Fish. Sci., 7:39-67.**
 3. Benfey, T.J. and Sutterlin, A.M. (1984) **Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). Aquaculture, 36:359-367.**
 4. Chourrout, D.; Guyomard, R. and Houdebine, L. M. (1986) **High efficiency gene transfer in rainbow trout (*Salmo gairdneri* Rich.) by microinjection into egg cytoplasm. Aquaculture, 51:143-150.**
 5. Collas, P. and Alestrom, P (1998) **Nuclear localization signals enhance germline transmission of a transgene in zebrafish. Transgenic Res., 7:303-309.**
 6. Devlin, R.H., Yesaki, TY., Donaldson, E.M., Du, S.J. and Hew, C.L. (1995) **Production of germline transgenic Pacific salmonids with dramatically increased growth performance. Canadian J. Fish. Aquat. Sci., 52: 1376- 1384.**
 7. Donaldson, E.M. (1997) **The role of Biotechnology in Sustainable Aquaculture. In: Bardach, J.E. (Ed.) Sustainable Aquaculture. J. Wiley and Sons.**
 8. Du, S.J.; Gong, Z.; Fletcher, G.L.; Shears, M.A.; King, M.J.; Idler, D.R. and Hew, CL. (1992) **Growth enhancement in transgenic Atlantic salmon by the use of an 'all fish' chimeric growth hormone gene construct. Biotechnol.,10:176-181.**
 9. Dunham, R.A.; Eash, J.; Askins, J. and Townes, T.M. (1987) **Transfer of the metallothionein-human growth hormone fusion gene into channel catfish. Trans. Am. Fish. Soc., 116:87-91.**
 10. Dunham, R.A, Ramboux, AC, Duncan, PL., Hayat, M., Chen, I.I., Lin, CM., Kight, K., Gonzalez-Villasenor, I. and Powers, D.A. (1992) **Transfer, expression, and inheritance of salmonid growth hormone genes in channel catfish, *Ictalurus punctatus*, and effects on performance traits. Molecular Marine Biol. and Biotechnol., 1 :380-389.**
 11. Dunham, R.A, Warr, G., Nichols, A, Duncan, PL., Argue, B., Middleton, D. and Liu, Z. (2002) **Enhanced bacterial disease**
-

- resistance of transgenic channel catfish, *Ictalurus punctatus*, possessing cecropin genes. *Marine Biotechnol.*, 4:338-344.
12. Entis, E. (1998) Taste testing at a top Canadian restaurant. *Aqua Bounty Farms*, 1:1-4.
 13. FAO (2005) **FAO Report on World Production of Fish - State of the World Fisheries and Aquaculture**. FAO, Rome, Italy.
 14. Farrell, AP., Benett, W. and Devlin, R.H. (1997) Growth enhanced transgenic salmon can be inferior swimmers. *Canadian J. of Zool.*, 75:335-337.
 15. Fletcher, G.L.; Davies, P.L. and Devlin, R.H. (1992) Genetic engineering of freeze-resistant Atlantic salmon. In: Hew, CL., and Fletcher, G.L. (Eds.) *Transgenic Fish*. World Scientific, London, pp. 190-208.
 16. Grinde, B. (1989) Lysozyme from rainbow trout *Salmo gairdneri* as an antibacterial agent against fish pathogens. *J. Fish Diseases*, 12:95-104.
 17. Gross, M.L.; Schneider, J.F.; Moav, N.; Moav, B.; Alvarez, C.; Myster, S.H.; Liu, Z.; Hallerman, E.M.; Hackett, P.B.; Guise, K.S.; Faras, A.J. and Kapuscinski, AR. (1992) Molecular analysis and growth evaluation of northern pike (*Esox lucius*) microinjected with growth hormone genes. *Aquaculture*, 103:253-273.
 18. Guillen, I.; Berlanga, J.; Valenzuela, C.M.; Morales, A.; Toledo, J.; Estrada, M.P.; Puentes, P.; Hayes, O. and de la Fuente, J. (1999) Safety evaluation of transgenic tilapia with accelerated growth. *Marine Biotechnol.*, 1 :2-14.
 19. Hedrick, P.W. (2001) Invasion of transgenes from salmon or other genetically modified organisms into natural populations. *Can. J. Fish. Aqua. Sci.*, 58:841-844.
 20. Hew, C.; Poon, R.; Xiong, F.; Gauthier, S.; Shears, M.; King, M.; Davies, P. and Fletcher, G. (1999) Liver-specific and seasonal expression of transgenic Atlantic salmon harboring the winter flounder antifreeze protein gene. *Transgenic Res.*, 8:405-414.
 21. Hostetler, H.A, Peck, S.L. and Muir, W.M. (2003) High efficiency production of germ-line transgenic Japanese medaka (*Oryzias latipes*) by electroporation with direct current-shifted radio frequency pulses. *Transgenic Res.*, 12:413-424.
 22. Hutchings, J.A (2000) Collapse and recovery of marine fishes. *Nature*, 406:882-885.

23. Hwang, G., Muller, F., Rahman, M.A, Williams, D.W., Murdock, P.J., Pasi, K.J., Goldspink, G., Farahmand, H. and Maclean, N. (2004) **Fish as bioreactors: Transgene expression of human coagulation factor VII in fish embryos.** *Marine Biotechnol.*, **10:211-221.**
24. Jank, B. and Gaugitsch, H. (2001) **Decision making under the Cartagena Protocol of Biosafety.** *Trends in Biotechnol.*, **19:194-197.**
25. Johnsson, J.I. and Bjornsson, B.T. (2001) **Growth-enhanced fish can be competitive in the wild.** *Functional Ecol.*, **15:654-659.**
26. Jonsson, E., Johnsson, J.I. and Bjornsson, B.T. (1996) **Growth hormone increases predation exposure of rainbow trout.** *Proc. of the Royal Soc. London. Series B. Biological Sciences*, **263: 647-651.**
27. Kapuscinski, A.R. and Hallerman, E.M. (1991) **Implications of introduction of transgenic fish into natural ecosystems.** *Can. J. Fish. Aqu. Sci.*, **48(Suppl. 1):99-107.**
28. Knibb, W.R. (2002) **Ecological Risk from Aquatic LMOs.** *Proceedings of Inter. Confer. "LMOs and the Environment"*, Raleigh-Durham, USA Nov. 2001.
29. Krasnov, A, Agren, J.J., Pitkanen, I.I. and Molsa, H. (1999) **Transfer of growth hormone (GH) transgene into Arctic Charr (*Salvelinus alpinus* L.) II. Nutrient partitioning in rapidly growing fish.** *Genetic Analysis: Biomolecular Engineering*, **15:99-105.**
30. Maclean, N. (2003) **Genetically modified fish and their effects on food quality and human health and nutrition.** *Trends in Food Science and Technol.*, **14:242-252.**
31. Maclean, N. and Laight, R. (2000) **Transgenic fish: an evaluation of benefits and risks.** *Fish and Fisheries*, **1: 146-172.**
32. Maclean, N., Penman, D. and Zhu, Z. (1987) **Introduction of novel gene into fish.** *Biotechnol.*, **5:257-261.**
33. Maclean, N. and Talwar, S. (1984) **Injection of cloned genes into rainbow trout eggs.** *J. Embryol. Exp. Morphol.*, **82:187.**
34. Maitland, P.S. and Campbell, R.N. (1992) **Fresh water fishes.** *Harper Collins, London.*
35. Martinez, R.; Estrada, M.P.; Berlanga, J.; Guillen, I.; Hernandez, O.; Cabrera, E.; Pimental, R.; Morales, R.; Herrera, F.; Morales, A.; Pina, J.C.; Abad, Z.; Sanchez, V.; Melamed, P.; Leonart, R. and de la Fuente, J. (1996) **Growth enhancement in transgenic**

- tilapia by ectopic expression of tilapia growth hormone. *Molecular Marine Biol. and Biotechnol.*, 5:62-70.
36. Muir, W.M. and Howard, R.D. (1999) Possible ecological risks of transgenic organism release when transgenes affect mating success: sexual selection and the Trojan gene hypothesis. *Proc. Nat. Aca. Sci. USA*, 96: 13853- 13856.
37. Muir, W.M. and Howard, R.D. (2001) Fitness components and ecological risk of transgenic release: a model using Japanese medaka (*Oryzias latipes*). *The American Naturalist*, 158: 1-16.
38. Nam, Y.K.; Noh, J.K.; Cho, Y.S.; Cho, H.J.; Cho, K.N.; Kim, C.G. and Kim, D.S. (2001) Dramatically accelerated growth and extraordinary gigantism of transgenic mud loach *Misgurnus mizolepsis*. *Transgenic Res.*, 10:353-362.
39. Ostefeld, I.A, McLean, E. and Devlin, R.H. (1998) Transgenesis changes body and head shape in Pacific salmon. *J. Fish Biol.*, 52:850-854.
40. Ozato, K.; Kondoh, H.; Inohara, H.; Iwamatsu, I.; Wakamatsu, Y. and Okada, I.S. (1986) Production of transgenic fish: introduction and expression of chicken delta crystalline gene in medaka embryos. *Cell Different. Develop.*, 19:237-244
41. Palmiter, R.D.; Brinster, R.L.; Hammer, R.E.; Trumbauer, M.E.; Rosenfeld, M.G.; Birnberg, N.C. and Evans, R.M. (1982) Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion gene. *Nature*, 300:611-615.
42. Rahman, M.A and Maclean, N. (1992) Production of transgenic tilapia (*Oreochromis niloticus*) by one-cell-stage microinjection. *Aquaculture*, 105: 219-232.
43. Rahman, M.A, Hwang, G., Razak, S.A, Sohm, F. and Maclean, N. (2000) Copy number dependent transgene expression in hemizygous and homozygous transgenic tilapia (*Oreochromis niloticus*). *Transgenic Res.*, 9:417-427.
44. Rahman, M.A, Mak, R., Ayad, H., Smith, A and Maclean, N. (1998) Expression of a novel piscine growth hormone gene results in growth enhancement in transgenic tilapia (*Oreochromis niloticus*). *Transgenic Res.*, 7:357-369.
45. Rahman, M.A.; Ronyai, A.; Engidaw, B.Z.; Jauncey, K.; Hwang, G. L.; Smith, A.; Roderick, E.; Penman, D.; Varadi, L. and Maclean, N. (2001) Growth and nutritional trials of transgenic

- Nile tilapia containing an exogenous fish growth hormone gene. *J. Fish Biol.*, 59:62-78.
46. Stevens, E.D.; Sutterlin, A and Cook, I. (1998) Respiratory metabolism and swimming performance in growth hormone transgenic Atlantic salmon. *Can. J. Fish. Aqu. Sci.*, 55:2028-2035.
47. Uzbekova, S., Chyb, J., Ferriere, Bailhache, I., Prunet, P., Alestrom, P. and Breton, B. (2000) Transgenic rainbow trout expressed GnRH-antisense RNA under the control of GnRH promoter of Atlantic salmon. *J. Mol. Endocrinol.*, 25:337-350.
48. Wilson, R.P. (1994) Utilization of dietary carbohydrate by fish. *Aquaculture*, 124:67-80.
49. WU, S. M.; Hwang, P. P.; Hew, C.L. and WU, J. L. (1998) Effect of antifreeze protein on cold tolerance in juvenile tilapia (*Oreochromis mossambicus*) and milkfish (*Chanos chanos*). *Zoological Studies*, 37:39-44.
50. Zhang, P.; Hayat, M.; Joyce, C.; Gonzalez-Villasenor, L.I.; Lin, C.M.; Dunham, R.A.; Chen, I.I. and Powers, D.A. (1990) Gene transfer, expression and inheritance of pRSV-Rainbow Trout-GH cDNA in the common carp *Cyprinus carpio* (L.). *Molecular Reproduction and Development*, 25:3- 13.
51. Zhao, Z.; Cao, Y.; Li, M. and Meng, A. (2001) Double-stranded RNA injection produces nonspecific defects in zebrafish. *Developmental Biol.*, 229:215-223.
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