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Genetically Modified Fish in Future Aquaculture: Technical, Environmental and Management Considerations

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Introduction and Objectives

Statistics on global fish and shellfish production show that fish farming represents about 15% of the global fish yields and is expected to exceed 20% by the year 2000. Capture fisheries have already reached their maximum sustainable yield, and this why any increase in seafood production can only be achieved through aquaculture. Increased productivity in aquaculture will be achieved through genetic improvement of species by selection breeding and, potentially, gene transfer technology.

Fish farming in ponds and dams was known in China 5,000 years ago and in Europe around 1,000 years ago. Trout farming started just before World War I, and salmon pen and cage farming began in Norway in the 1970s. About ten years later, a national breeding program was developed, becoming the starting point of this new activity (Gjedrem et al. 1988). After six generations of selection breeding a Norwegian salmon was produced that was 50% bigger than its ancestors from the Norwegian rivers. Later, the Aquaculture Research Institute (AKVAFORSK) successfully transferred the Norwegian salmon breeding program to tilapia farming in the Philippines and carp farming in India. The history of farmed salmon makes it one of the last species to be domesticated together with a few other cultured fish species, although fish culture as such has been known for thousands of years.

Efforts are now underway to domesticate new species for aquaculture and to meet the global demand for increased food production. In order to optimize results in new breeding programs, genetic engineering and genetically modified organisms (GMOs) offer new possibilities. The objectives of this paper are to discuss technical, environmental and management considerations regarding the use of GMOs in aquaculture.

Technical Aspects

Genetic Improvement of Fish by Gene Transfer

An organism which receives a new gene transferred to its genome is called a genetically modified organism (GMO) or a transgenic organism. Transgenes can originate from phylogenitically distant organisms or from the same species. This technological development makes the improvement of fish and other aquatic organisms possible today (Fletcher and Davies 1991; Powers et al. 1993; Aleström 1995; Hew et al. 1995).

There are various methods for transferring genes to fish. Most common, and so far most successful, is microinjection. DNA microinjection requires the use of a capillary pipette with a tip measuring 0.005 mm in diameter to inject 0.00000025 ml of DNA solution into fertilized fish eggs. The development of so-called embryo stem cells will allow the qualitative and quantitative insertion of a given gene through homologous recombination (today only working in mouse).

Most recent reports on the production of transgenic fish species describe the transfer of “all fish” gene constructs, potentially using DNA sequences derived from the same, or closely related, species. This process does not involve adding new gene sequences to the genome but multiplying the number of copies of already existing genes. However, changes are often introduced to control gene expression by fusing promoters non-specific to the gene coding sequence.

What Species and What Traits?

A number of species are targeted for gene transfer experiments and can be divided into two main groups: fish used in aquaculture, and model fish used in basic research. Among the major food fish species are carp (*Cyprinus* sp.), tilapia (*Oreochromis* sp.), salmon (*Salmo* sp., *Onchorryncus* sp.) and channel catfish

(*Ictalurus punctatus*), while the zebrafish (*Danio rerio*), medaka (*Oryzias latipes*) and goldfish (*Carassius auratus*) are used in basic research. In addition to food production and research, carp and goldfish are widely used as ornamental fish. Not only fish, but also other seafood species important in aquaculture like shellfish and shrimps, are now coming into focus for research on gene transfer mediated improvements.

In classical breeding programs, economically important traits like growth rate and food conversion ratio (FCR) have been ranked the highest, aiming at an increased food production. Several other traits of economic importance could not have been improved without genetic improvement. The pink color of salmon is obtained by adding synthetic carotenoids into food pellets. The lack of disease resistance which required heavy use of antibiotics until recently is now controlled through the development of new vaccines.

Today gene transfer technology only applies to copying single or a few gene traits. In addition, candidate genes must be characterized at the molecular level. Only a limited number of traits and genes fulfill both criteria and thus are candidates for genetic improvement. This justifies further research on genes controlling disease resistance, aggressive behavior, flesh color, etc.

Growth control through growth hormones (GH) belongs to the area of vertebrate physiology which is well understood. In the early 1980s the successful transfer of human and rat GH genes to fertilized mouse eggs which resulted in fast-growing transgenic mice has since stimulated many groups to achieve genetic improvements in different farm animals by genetic engineering. Several laboratories now have GM fish with increased growth performance caused by extra copies of GH genes. Transgenics with a growth performance up to 30 times higher than the average of non-transgenic siblings have been reported (Hew et al. 1995; Devlin et al. 1995).

Cold resistance achieved through the use of an antifreeze protein (AFP) is another trait which is the focus of a much research efforts. AFP genes have been transferred from the genome of the ocean pout (*Macrozoarces americanus*) to the salmon and to goldfish with the aim of introducing the cold-resistance trait (Hew et al. 1995). Recently, fish AFP genes have also been transferred to plant species in order to make the recipient plants cold resistant.

There are two reasons for developing sterile fish. Onset of sexual maturation with gonad development results in both loss of weight and meat quality in salmon. Secondly, sterile escapees cannot reproduce in the wild, which is an efficient way of biologically segregating species. Sterile fish are now produced through chromosome manipulation which leads to triploid, often monosex, fish. One way of producing a transgenic sterile fish is to inhibit production of the main sex hormone GnRH, by transferring special anti-GnRH genes (Aleström et al. 1992). Other ideas of introducing biological barriers against uncontrolled reproduction are discussed below.

One special category of GM animals is called “bioreactors” because the animals are used exclusively for the production of a certain protein. Fish have been suggested as candidates for this category of organisms, along with mammals and plants.

Environmental Issues

Are GM Fish Safe to Eat?

GM fish as such do not represent any health hazard. Of major importance for any health risk evaluation is the character of the gene, the gene product it encodes and the resulting phenotype. In addition, it is important to ensure that the insertion of a new gene has not affected an endogenous gene or had other pleiotropic effects.

Most of the existing GM fish prototypes have received extra copies of their GH genes, resulting in only moderately raised levels of circulating GH. GH is a protein hormone which is degraded along with all other food protein. Meat from fish modified with GH is regarded as completely safe for human consumption. In addition, food quality control includes taste, appearance, color, texture etc. parameters which are important for commercial success, but not necessarily for health safety. Finally, consumers will decide if they will accept GM food or not.

Natural Ecosystems

It is generally accepted that inadvertent release of farmed fish into natural ecosystems should be avoided, especially if the fish has been genetically modified. The physical containment of fish farms has proven to be efficient only in land-based facilities. Seapens are more prone to accidents because of extreme weather conditions and on several occasions, the accidental release of large number of farmed salmon has been reported. In many Norwegian rivers farmed salmon are now established, and reproduce and compete with the local strains, although they are generally less fit (Hindar, pers. com.).

Biosafety and Risk Assessment

A commercially available, transgenic fast-growing fish is already marketed worldwide by the US company A/F Protein. Plans to introduce it into Scottish fish farms have recently caused much debate in Europe. In Cuba, plans for marketing transgenic fast-growing tilapia were presented at the congress Biotecnologica Habana '95. The establishment of a homozygous line of GM Atlantic salmon takes a minimum of 13 years from microinjection until the GM fish can be introduced into a breeding program (OECD 1993). For species like tilapia and carp, which have shorter life cycles, the required time frame will be shorter. Finally, before production can begin it will be necessary to seek approval from the environment and health authorities.

In addition to practical restrictions and limitations, general ethical and animal protection concerns demand that the effects of transgenes and transgene product on animal health be adequately addressed before any commercial fish farming can be initiated. If the animal's health is not negatively affected by transgenes or transgene product, it can be inferred that GM fish do not represents health hazards for human consumption (Berkowitz and Kryspin-Sørensen 1994). In the case of fast-growing GH fish, symptoms similar to acromegaly can be observed in some of the animals with higher growth levels, although the general impression at present is that the majority of transgenics are healthy.

Risk assessment of GMOs is given priority both by national and international institutions engaged in biotechnology development. In August 1994, the First International Symposium on Sustainable Fish Farming was held in Oslo, Norway (See Aleström 1995). During this conference "The Holmenkollen Guidelines for Sustainable Industrial Fish Farming" were discussed and approved. Recommendation no. 14 requested that "Farming of transgenic or other genetically manipulated fish should not be undertaken until internationally agreed safety and ethical criteria have been established". ICES definition of GMOs included both transgenics and fish with modified chromosome sets, but not fish which have been improved by classical breeding (ICES 1996). In the discussions, biological containment schemes were suggested as an important complement to the physical barriers.

Biological containment is well-known from biosafety restrictions of bacteria used in recombinant DNA research and industrial applications. These often include mutations in genes of metabolic pathways, making the organism dependent on having the missing metabolite supplemented in the growth medium. Biological containment in the context of aquaculture includes sterile triploid fish or sterile transgenic fish carrying anti-fertility genes tailored into their genomes (Aleström et al. 1992; Donaldson et al. 1993). As well as establishing and reproducing themselves in wild biotopes, escaped salmon have in several cases spread disease to wild populations. This problem cannot be solved by fish sterility, which is why the introduction of suicide genes allowing survival only in capture conditions has been suggested. Triploid fish technology is a simple cost-effective method of producing sterile fish which is in use in many countries. The disadvantage is that every egg must be treated and that the extra set of chromosomes represents a cellular content of nucleic acids 30% higher than in normal fish. One EU Biotechnology research project, titled, “Biological containment of transgenic fish and risk assessment of inter-species gene transfer”, aims at making GM sterile fish by expressing anti-fertility genes which inhibit the production and action of the chief gonadotropin releasing-hormone (GnRH) (Aleström et al. 1992). The advantage would be that sterility would be introduced as a stable hereditary trait, where only selected broodstocks are rendered fertile through hormone therapy. These GM sterile fish would also carry the normal chromosome number and DNA content.

Risk assessment in cases of deliberate or accidental release of transgenic fish depends on a number of factors: (i) The species which is released and the biotope it is released into, (ii) the character of the transgene and the new phenotype, (iii) the general fitness of GMOs versus wild populations and finally (iv) the number of released GM fish. There is quite a lot of experience from the introduction of exotic species into the environment which is helpful for assessing the risks associated with the release of GMOs, and every new GMO could, in a way, be regarded as a novel exotic species. Although this can be considered as a general guideline, there is no knowledge on the performance of GMOs and because this type of knowledge is critical, a careful approach is warranted, i.e., a case by case and stepwise approach, starting with physically contained on-station trials and moving to small-, medium- and large-scale on-farm trials. A case by case approach corresponds the calculated risk which can be made from evaluating the above factors. Evaluation at each step will indicate if moving to the next is acceptable.

International Research Collaboration and Sustainable Development

In several areas ranging from fish culture economy to basic research in biology and medicine, transgenic fish technology is expected to play an important role in the future. Improved growth in GM fish is up to now the most successful example of genetically modifying a trait of economic importance, achieved in several fish species in laboratories in both developed and developing countries. A growing concern to prevent farmed fish (both GM and non GM) from escaping and competing with wild strains now calls for the use of sterile fish which cannot reproduce and interbreed with wild populations. Recently, disease problems forced the Norwegian salmon farming industry to use large amounts of antibiotics. The development of new fish vaccines has largely solved the problem. The risk of disease added to the cost of vaccination makes potential disease resistance through gene transfer a high-priority research strategy. Increased and sustainable development through the use of GM technology can be achieved on the conditions that large investments be made in basic and applied research efforts that are critical to improve understanding on how complex traits can be controlled by single genes. These research efforts should be carried out in international collaboration so that basic expertise can be developed in countries using the technology, and so that the technology can be developed in accordance with the needs and socioeconomic conditions of the user nation or region.

Conclusions

GM fish offer new potential for increased production of cultured organisms. This technology allows the introduction of new traits, or the improvement of old ones, in a way that is impossible to achieve with conventional breeding methods. Examples of genes with commercial potential are those which control growth, disease resistance, cold tolerance, sexual maturation, food quality and preservation.

GM fish food does not pose any major risk to health. GM food safety depends on the nature of the transgene, the transgene product and the new phenotype. Ethics and animal protection concerns allows the development of healthy and safe fish only.

Environmental protection calls for efficient physical containment along with biological containment in order to minimize the effects of the release of farm animals. Improved disease control will contribute to increased productivity and to environmental protection in case fish escape from fish farms.

Since aquaculture includes both marine and freshwater species, it can be developed as a new food production strategy for most countries. To avoid technology transfer failures, it is important to adapt systems to existing conditions in terms of regional and local needs. This calls for international research collaboration at the regional and local level which can develop appropriate expertise for implementing the new technologies.

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