

Applicability of gene transfer into the germinal line in fish culture

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Introduction

Transgenic organisms are one subset of genetically-modified organisms (GMOs) as defined by the European Union. Strains of transgenic fish have been developed in Asia, the Americas and Europe nearly 20 years ago, but many of the strains were not retained and commercial development of strains for aquaculture in Europe is currently minimal.

The failure of transgenic fish to be implemented into aquaculture has arisen for two main reasons: 1) significant social concern regarding food safety and environmental impacts which may arise from their use; and 2) despite encouraging data from laboratory studies (Fig.1, comparing normal salmon to those transformed with a growth hormone gene construct), a clear demonstration has not been made regarding the economic benefit of transgenic fish relative to existing strains which have been genetically improved by traditional methods, such as selective breeding. Nevertheless, internationally, research has continued in Asia and the Americas examining the commercial aspects and risk assessment of transgenic fish, and one company, Aquabounty in the USA, is applying to the US FDA for approval for marketing of the transgenic fish. This regulatory decision has been slow to be achieved, one speculates because of the complexity of the risk assessment task which accompanies such a decision. If approved, the application of these strains in large markets which do not require labelling has the potential to significantly shift the economics of salmon farming globally and could stimulate other jurisdiction to consider their implementation and to mediate pressure on international trade organizations which can regulate the sale of transgenic fish. Thus, scientific information needed to assist regulatory decisions is urgently required.



Fig. 1. Normal (left) and GH-transgenic salmon (right) of the same age.

Due to the current state of uncertainty associated with the use of transgenic organisms, coupled with their potential to assist in the efficient production of food at the global level, new knowledge is urgently required to clarify some of the major outstanding issues. Thus, Workpackage 1.3 of Workshop 1 of the EU Genimpact project has posed several questions to stimulate discussion of current issues associated with the use and safety of transgenic organisms. In the present context, transgenic organisms were considered as those containing novel gene constructs (i.e. homologous or heterologous DNA arranged in non-native forms) that had been stably integrated into the germline of fish. Several main areas were considered: 1) definition of genetically-modified organisms (GMOs); 2) issues associated with food safety; 3) environmental risks and reliability of experimental assessments of transgenic fish for hazard assessment; and 4) approaches for enhancing the biocontainment of transgenic fish.

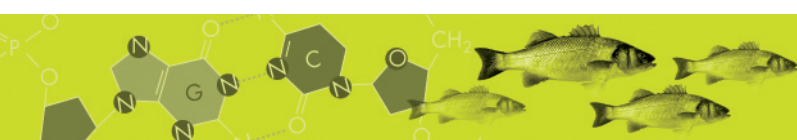
From the discussion, research priorities have been suggested (see end) which could guide the acquisition of new knowledge and technology for the development and containment of transgenic technology.

Summary of responses to questions

1) *Applicability of the substantial equivalence principle for the marketing of GH-transgenic fish*

a) *Is it appropriate that the current definition of the term “genetically modified organism (GMO)” be process-based rather than product-based?*

In the EU, a process-based regulatory framework exists for evaluation of GMOs, which differs fundamentally from other global jurisdictions [*i.e.* Canada (*e.g.* Canadian Environmental Protection Act, CEPA 1999), USA and Australia] where regulations are based on characteristics of the “product”. The rationale for using a product-based regulatory framework stems from the logic that it is the characteristics of the organism or its by-products which can result in health effects or environmental consequences. The nature of the process that generated two *otherwise identical* products does not influence the consequences of that product and therefore should not form the basis of regulatory scrutiny. Should identical strains produced through natural and anthropogenic means be regulated differently? Arguments for using a process-based regulation are derived from the consideration that certain processes, including transgenesis, may yield specific types of products which carry genetic changes (and thus create uncertainty and risk) that other approaches do not (such changes may or may not be identifiable with current technology; *e.g.* hypothesized trans effects on genes distant from the site of transgene insertion). It was noted that many natural processes (*e.g.* selective breeding, mobilization of transposons, etc.) also can yield many genetic changes which are not easily detected with current technology, but which may influence phenotype in both complex and simple ways. Significant discussion occurred within the group regarding an appropriate definition of what constitutes a genetically-modified organism. The definition ranges widely among researchers, from changes induced by altering the frequency of natural alleles in selected populations, to polyploidy and hybridization, to the transfer of non-integrated DNA into somatic cells of fish, or, in the strictest definition, to only those organisms that have been permanently transformed in their germ lines with DNA from a heterologous source.



GMO has been defined by the EU as «Genetically modified organism (GMO) means an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination» (see below). In some jurisdictions, such as Canada, transgenic organisms are covered under regulation of “novel” organisms, *i.e.* those which have characteristics that have not previously been introduced into nature. It is recommended that further clarification of the use of the terms GMO (Genetically modified (or manipulated) organism), GEO (genetically engineered (or enhanced or enriched) organism), and novel organism, be undertaken in the EU.

A discussion also occurred regarding the use of substantial equivalence as an approach for regulating GMOs. Arguments for the approach suggest that, for example, expression of a host gene sequence (such as isospecific or homospecific growth hormone) may only quantitatively alter the characteristics of the organism, and does not affect the organism qualitatively. Arguments against this approach cite the potential of undetected changes to be generated by transgenesis that could yield novel protein products that may induce health or ecosystem effects, and that full analysis of the transcriptome and proteome are required to assess whether changes have occurred (it is noted that full transcriptome and proteome analyses are not yet possible for fish).

An alternate approach to using substantial equivalence is to adopt food safety evaluations used for all novel foods including those for organism which have altered level of a host protein, or a novel protein not normally present in the organism. Such tests would include allergenicity tests and multi-generational rat feeding trials followed by pathology tests and health status evaluations to search for *significant* effects on mammalian health. Such evaluations are likely to detect the vast majority of *acute* effects that have been hypothesized to be able to arise from transgenesis. Only long-term use of any novel product in a recipient population is likely to discover more subtle consequences, if they exists (analogous to the discovery of side-effects in most approved drugs following approval and wide-spread use in the population).

b) Is it appropriate that the current concern about GMOs be focussed on genetic changes rather than phenotypic changes?

Regulations should be based on the molecular, physiological and behavioural phenotypes associated with the organism, and most importantly how these characteristics differ from those found in wild type or other reference strains. Such differences then need evaluation with regard to food safety requirements and ecological consequences using the best methods available. Genetic changes are important with respect to how they may influence expression (*e.g.*, through position effects arising from characteristics of the specific site of integration) and stability (*e.g.*, is the site prone to deletion due to flanking repetitive elements, or alteration in copy number arising from changes in construct number which are organized in tandem arrays ?).

c) Since transfer of multiple foreign genes can spontaneously occur by introgressive hybridization, why introgressed fish are not to be considered as natural GMOs?

In many cases, hybrids are not novel to the environment, having arisen through natural interbreeding between species. Such hybrids contain genes and gene arrangements that have previously undergone natural selection in the wild. Although their genes now may be expressed in a novel nuclear environment, natural hybrids have had the historical chance to transfer genetic information. The consequences



of such exchange have further been subjected to natural selection. In cases where larger numbers of such hybrids are anticipated to enter an ecosystem through release or escape, then consideration of the effects of backcrossing with the parental species or further hybridization events should indeed be considered (*i.e.*, to evaluate the potential to significantly alter the population genetic structure of the resident population(s)). If the hybrids involve species that have not naturally hybridized, or they have the potential to enter novel environments, then careful examination of the characteristics of the organisms should be undertaken as would be performed for any novel organism (*e.g.*, introduced nonindigenous species).

- d) *Why were GMOs not regulated according to the same practice as at least fifteen fast-growing, fertile interspecific hybrids, which are currently admitted for fish culture despite the risk of back-crossing?*

See c) above. For some hybrids, there may already be significant information available regarding their fitness in nature, which can assist with regulatory decisions about their potential consequences following introduction.

- e) *Is an aquatic organism bearing a construct encoding an isospecific protein eutopically expressed still to be defined as a GMO or rather as a “genetically enriched organism (GEO)”?*

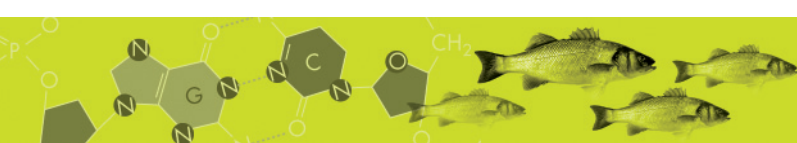
The type of protein expressed from a gene construct is only one part of the genetic information contained within it. Other critical genetic characteristics of the construct include regulatory changes which influence developmental timing of expression, tissue specificity, and quantity of the gene product. Each of these features has the potential to alter an organism significantly from its wild-type state. Altered tissue-specific expression can qualitatively influence the characteristics of an organism (*e.g.* from a food safety perspective, expressing a protein in muscle that is normally only found in liver, may alter the food characteristics of the flesh for human consumption). Altered levels of a protein can lead to downstream effects of other protein regulated by the transgene product (*e.g.* GH is known to stimulate IGF-I, a highly conserved vertebrate growth promoter) or may affect physiological process (*e.g.* thyroid hormone metabolism) or behaviour (*e.g.* GH is known to be a potent stimulator of feeding behaviour, which has a strong effect on the fitness of organisms in nature).

- f) *Are sterile transgenic fish, that cannot vertically transmit their transgene, still to be classified as GMOs? Is transformation of a functional germ line not required?*

Yes, such organisms are still very much genetically modified in that they contain novel genetic information in their somatic and (in this case non-functional) germline tissues. Their entry into nature may still result in effects on ecosystem function over their lifetime. However, estimating such direct effects is vastly easier than the case where sexual reproduction can occur and result in the generation of new transgenic individuals in nature.

- g) *Do fast-growing fish bearing an isospecific GH-encoding construct satisfy the substantial equivalence principle for human consumption, because their protein profile has only been quantitatively modified? Should their sale still be subjected to stricter regulations than normal fish?*

See a) and e). Quantitative changes can lead to qualitative changes, and to alterations in physiology and behaviour outside of the norm observed in the species. The effects



of overexpression depends very much on whether the direct and downstream effects have altered the product quality from that found within the normal range. If GH levels (amount produced, not the amount present in plasma) have not been raised beyond a level that is found within the normal range of already-consumed strains of the same species, then substantial equivalence may be considered. Arguments against substantial equivalence suggest that unidentified alterations may also have accompanied the known transgene insertion, and that undetected health/ecosystem impacts may well also exist. Empirical evidence demonstrating such hazards need to be carefully documented and scrutinized to ensure that the precautionary principle is not unnecessarily applied by infinite risks hypotheses.

2) *Environmental safety of non-sterile transgenic fish and possible scenarios of transgene contamination of wild fish populations*

a) *How urgent is the approval of GH-transgenic fish for fish culture to meet future aquafood demands?*

Discussion included which species are important to assist in global food production, where the food should be produced, and whether the technology should be developed in the EU to be provided elsewhere for application. The workshop attendees felt that transgenic salmon were not useful for this purpose, and that genetic engineering of this species primarily has the target of enhancing commercial efficiency of production and commercial benefit, and would be unlikely to significantly assist in providing animal protein for human consumption in developing nations. Other species such as tilapia and carp were recognized to have significant potential to assist in world animal protein production, and that enhancement of strains of these species by transgenesis could be of use. Characteristics felt important included enhancing food utilization (feed conversion efficiency), enhancing disease resistance, modifying the product characteristics (i.e. to make a healthier product with superior essential lipids and amino acid profiles), and improving abilities to utilize plant-based feed ingredients. Utility of transgenic technology also urgently requires better assessments of the benefits of GH transgenic fish under production conditions. Despite the apparent gains observed in laboratory trials of transgenic fish (particularly with GH), it was noted that few data currently exist regarding their utility in aquaculture environments (*vs.* the lab), and that comparisons to the best available domesticated strains is critical to evaluate their efficacy for enhancing food production. The use of fish species which can utilize vegetarian diets was also noted to be beneficial to those requiring fish/animal meals and oils which are now becoming very limiting.

b) *Should the transfer of a construct allowing allopatric invasiveness be prohibited at all times similarly to the introduction of alien species?*

Many constructs can be anticipated to alter the biogeographical range of a species, mediated through physiological enhancement of characteristics (*e.g.* ability to use nutritionally inferior prey items found in distant habitats) or addition of novel traits (*e.g.* antifreeze proteins which can confer enhanced cold tolerance and thus ability to invade new territories). In few cases, whenever the exact ability of a transgenic organism to possess enhanced host range is known, decisions regarding its risk to the environment should be determined from empirical studies examining its phenotype (*e.g.* thermal tolerance studies, nutrition trials, etc.).

c) *Since private companies around the world are developing at least 20 species of transgenic fish and shellfish, should the choice of species be profit-oriented for private enterprises and relief-oriented for public agencies?*



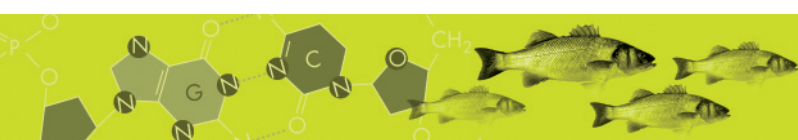
Both public and private research organizations can contribute both to private enterprises and global assistance objectives. Some felt that public funding should not be used to support transgenic research of any form, whereas others felt that such support could be used to develop technology which could be provided to both sectors. Others felt that this was not a question that was appropriate for science to decide. Political institutions and social policy should rather influence or make these decisions, that may vary among jurisdictions and over time. It was also noted that “private” transgenic organisms could be licensed by public entities to provide for relief purposes.

- d) *How realistic is the “Trojan gene hypothesis” by Muir and Howard (1999) assuming that GH-transgenic escapees with a mating advantage due to their bigger size but affected by inferior environmental adaptation might cause the extinction of wild populations?*

The Trojan Gene (TG) hypothesis was acknowledged to be theoretically possible under specific stable conditions. A parallel was drawn to an extinction vortex proposed to occur from hybridization of wild fish with less fit domesticated genotypes. A difference noted with the TG hypothesis is that this scenario predicts that entire species extinction may arise by the introduction of, theoretically, a single transgenic individual (with the appropriate pair of opposing fitness traits) into a population, whereas an extinction vortex caused by interbreeding between wild and domesticated strains requires either sustained introduction of domesticated stock or the introduction of a very large population of domestic strain, which effectively eliminates the wild population genetics of the species. In the absence of a sustained input of a domestic stock, data are unavailable to determine whether such impacted populations may re-establish their natural allele frequencies which had been achieved through natural selection for high fitness in the wild. Factors which would limit the likelihood of the TG hypothesis from becoming a reality in nature include: 1) the extinction effects only occur under a restricted range of fitness conditions; 2) natural selection of background genetic variation will shift fitness values over time, resulting in diminution of the negative fitness trait away from that required for a TG effect; and 3) to date, GH fish do not always show enhanced body size and mating advantage relative to nontransgenics.

- e) *How realistic is the assumption that the growth enhancement promoted by GH-construct transfer would also be associated with greater predatory capacity in transgenic escapees in the wild?*

GH transgenic fish that have been tested display significantly enhanced feeding behaviour and prey capture rates. In trials where transgenic and nontransgenic fish are in competition, transgenic fish consistently acquire food resources preferentially over controls. In nature, however, enhanced feeding ability does not necessarily translate into enhanced fitness, since fish in many cases must trade off the benefits of foraging activity with the risk of being subjected to predation themselves. Thus, growth rates of fish in nature are not often maximal, but rather have been selected to provide maximum fitness. GH transgenic individuals have had their feeding behaviour forcibly altered away from a naturally-selected, presumably, optimum. In semi-natural environments, where transgenic fish are foraging for natural prey items, it has been found that they succumb to predation mortality at a greater rate than controls and thus display lower fitness. It has also been found that populations containing GH transgenic are less able to withstand periods of food shortage, resulting in population crashes. Developmental rate has also been found to affect



the fitness of transgenic fish, such that early emergence from natal redds has been shown to result in preferential predation mortality.

- f) *A non-indigenous species that has become naturalized after its introduction (e.g. Nile tilapia or its hybrids) is a better candidate for gene transfer than an indigenous species?*

A naturalized species may have a better chance of survival in nature and thus causing effects on other organisms. Using highly domesticated strains with poor natural fitness (but high cultured fitness) that have no counterparts to breed with in the vicinity may be useful for containment. The use of monosex strains or sterile hybrids also could provide high level containment for some species, where no conspecifics or other related species are present in the ecosystem to breed with.

- g) *Are fish feeding at the bottom of the trophic chain (phytoplanktonophagous, vegetarian) better candidates for increasing world aquafood production by means of gene transfer than carnivorous species? Is the issue of sustainability relevant to transgenesis application in fish?*

Farming of fish that have high production efficiency should be the goal for world fish production, using feed ingredients derived from as close to sunlight as possible better (*i.e.* plants, algae). Such species may have a greater potential to alter food webs, however, and cause environmental damage, depending on the exact species they are capable of harvesting from the environment. This must be assessed on a case-by-case basis.

- h) *Why is transfer of foreign non-coding sequences (eg. satellite DNA) into wild conspecific populations by crossing with domesticated escapees not considered as a gene transfer? The phylogenetic record of the wild population is certainly perturbed.*

If this transfer can occur through natural hybridization, then the resulting organism is not novel to the environment. If it is something not seen before in nature, then some jurisdictions would consider it a GMO (or novel) which would require evaluation. Thus, the current definition of GMO by the EU may require some modification.

- i) *Should genetic mutations induced by genotoxic substances discharged with industrial effluents in inland water bodies or marine coasts be regarded as true “genetic modifications” and the affected organisms classified as covert GMOs? What about the ecosystemic damage due to the transgenerational increase of the genetic load in wild populations?*

Yes, all genetic changes that may produce a novel phenotype should be examined. But this would be a very large task to undertake. Disruptions of the natural population genetic structure of fish is an important impact that needs to be evaluated during a risk assessment.

3) Feasibility and reliability of recommended field tests to assess the environmental safety of transgenic fish

- a) *What qualifications should the experts in charge of developing legislation for transgenic aquatic organisms have?*

Research experience in biological sciences (genetics, molecular biology and ecology), and experience in Risk Assessment methodology and Decision Analysis, as well as legal experience are required to provide a reliable risk assessment.



b) How should field tests be carried out to avoid dispersal of tested transgenics?

Fish released to natural ecosystems are essentially unrecoverable. Thus, if the result is subsequently determined to be detrimental to the environment (e.g. Nile Perch in Lake Victoria), it is unlikely that recovery will be possible. Thus, the use of laboratory apparatus, semi-natural mesocosms, and modelling are alternatives that can provide useful data of phenotypic effects. An alternate approach is to utilize surrogate systems to mimic transgenic animals. For example, slow-release formulations of fish GH can be implanted into fish which can be released to nature without risk of sustained genetic consequences. Similarly, although as yet untried, individually-verified triploid sterile animals introduced into contained aquatic natural environments may be developed with minimal potential for long-term environmental consequences.

c) Is it legitimate to extrapolate results about competitiveness of transgenics in a secluded biotope to the different situations of open environments?

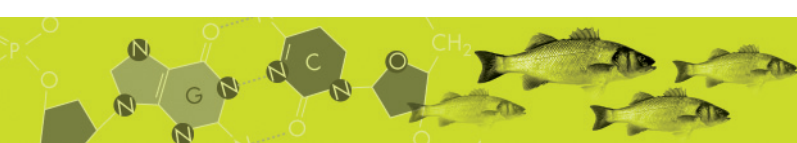
Results to date examining the traits of transgenic and control animals in the laboratory have revealed that there are very strong environmental effects on phenotype (phenotypic plasticity). More importantly, this plasticity is subject to genotype by environment (GxE) interactions, which results in nonlinear reaction norms for trait character across different environments. Such results strongly suggest that data acquired in the laboratory, even under the most complex apparatus that can be developed, are likely to fall short of accurately representing the full spectrum of environments that a transgenic fish is likely to encounter in nature. Certainly all effort should be made to cover a broad range of conditions, from which sensitivity analysis may be used in models to assist in predicting critical controlling parameters. However, definitive identification of the true fitness of a transgenic animals and its consequences cannot easily be determined from lab studies. Due to these uncertainties, biocontainment strategies are urgently required.

d) How transparent can a field test be carried out within the internal facilities of a private company? What if activists opposing GMOs infiltrate a public facility for transgenic tests?

Ideally, all pertinent risk assessment information should be published in the peer-reviewed literature. There is also a benefit of public research programs and model strains of transgenic fish which allow (open) access to perform studies (in contained facilities) and publication of information regarding the benefits and risk of transgenic fish technology. Hence, everybody can (openly) evaluate and challenge the GMOs using scientific methods.

e) Is the requirement of field tests actually a demand for a never-ending sequel of expensive trials of dubious significance, prone to conflicting results and exposed to the enforcement of a strong precautionary principle with oppressive bureaucracy? Or are they absolutely necessary?

To obtain *true fitness and consequence* data, field tests are the only possible approach. If contemplated, field trials should be performed only after lab and semi natural mesocosm studies assessing phenotype and consequence have been performed, and should be conducted only with biologically-contained animals (which limits the level of impact that can be studied). Currently, the uncertainty associated with transgenic fish fitness and consequence in nature are too great to support field trials, given the inability to recover such fish should an introduction with negative consequences ensue.



- f) *Should field tests be conveniently replaced by straightforward anatomical, histological and functional fertility tests, implying that all transgenics must be proved as reproductively incompetent?*

Sterility can be confirmed in individual fish using triploidy analysis (*i.e.* flow cytometry of blood cells). This method is currently used successfully to introduce sterile grass carp for weed control in the USA. The application of triploidy testing for aquaculture is not likely to be economically viable on a large scale due to the requirement for handling and analysis of each fish. Alternative methods of biological containment are required. Private companies may accept this requirement, which would confer them full market control on their product without the risks of being sued at later times for unforeseen transgenic contaminations. Sterilization technology provides convergence of ecological and economic benefits associated with the application of transgenic fish.

4) *Technical approaches to achieve full biological containment of farmed transgenic fish*

- a) *Is the transfer of a construct encoding anti-sense mRNA for GnRH an adequate measure to ensure complete sterility during growout of transgenic fish?*

To date, this approach has resulted only in partial sterility. Other approaches including RNAi and targeting all GnRH loci in the genome, may prove to be more effective. Full restoration of maturation and fertility by treatment with exogenous GnRH peptide must also be demonstrated in the sterile strains for the technology to be effective.

- b) *Can direct and indirect techniques for triploidy induction secure complete sterility of transgenic fish and shellfish?*

Temperature and pressure shock-induced triploidy in finfish does not yield 100% triploid populations of animals. Experiments on large numbers of transgenic coho salmon have shown that while high levels of triploidy can be achieved (99.8%) with pressure shocking, varying the treatment conditions (*i.e.* temperature, shock pressure, timing, and duration, age of eggs, time post fertilization) does not eliminate diploid exceptions for treated groups. Given the large number of fish which can escape from aquaculture facilities, even a frequency of 0.2% could allow the introduction of a significant number of fertile animals into natural ecosystems. The use of tetraploidy may enhance triploid production; however, it should be noted that tetraploid strains generally have impaired viability, and evidence has been presented indicating that unreduced gametes can arise from tetraploid strains (which would result in pentaploid rather than triploid offspring). Nevertheless, this is a promising area of research which should be further explored.

- c) *Are sterile fish hybrids obtained by intergeneric hybridization of transgenic pure species safe candidates for mass culture? Should distant hybridization be associated with triploidy induction to produce sterile transgenic allotriploids with adequate biocontainment?*

Such hybrids if reliably sterile as diploids, or inviable as diploids but sterile as triploids, could be of extreme value, particularly if coupled with triploidy. This approach could provide backup containment if the triploidy induction process failed. This approach would need to be evaluated on a large scale to examine rare meiotic events which may yield gynogenetic and/or aneuploid offspring.



d) Should transgenic fish be raised only in closed recirculating systems?

If biological containment cannot be achieved, then effective containment can be achieved using land-based facilities. The economic benefit associated with the enhanced production trait of the transgenic strain would need to offset the significant investment required for land-based aquaculture. Protection against predictable catastrophes (hurricanes, floods, earthquake) also needs to be considered in this case (*e.g.* carp escapes from ponds in Europe).

e) Are techniques available for crippling transgenic fish in order to make them unable to survive in the wild?

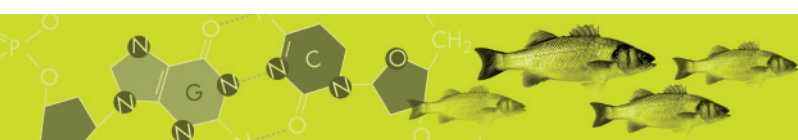
There are a variety of genetic and molecular biology approaches which can be developed to enhance biological containment. Developing knockout technology (*e.g.* site directed mutagenesis, TILLING, and RNAi methods) could allow development of strains which are dependant on supplemental compounds for survival and or reproduction. These techniques are in their infancy, and should be supported since they have the ability to provide solutions for containment not only of transgenic fish, but also of nonindigenous species and selectively-bred native strains. If transgenic approaches are utilized, these too would require separate risk assessments.

f) Has any country provisionally approved transgenic fish for human consumption?

The group is unaware of any transgenic fish being used in aquaculture at this time, despite several strains apparently ready for deployment should approval be granted by regulatory agencies.

Research Priorities

1. Research examining the novelty (physiological, morphological, and behavioural) of different forms of genetic modification using molecular tools such as DNA microarray, proteomics and QTL mapping are required to reveal the underlying basis of phenotypic differentiation among strains. For example, are growth-enhanced and domesticated fish similar enough to be regulated in the same way?
2. Knowledge on how genetic and epigenetic changes influence the expression of transgenes and thus the phenotype of the modified organism is needed to evaluate the long-term stability of GMOs, and also their response to environmental variables (phenotypic plasticity and GxE effects). Understanding phenotypic plasticity of traits, and most importantly genotype by environment interactions, is at the key of evaluating the reliability of risk assessments. It will hardly be possible to provide reliable phenotypic data on different transgenic and wild type strains, which vary under different environmental conditions, for robust predictions in nature.
3. Hybridization should be examined for its ability to create novel phenotypes not previously present within an ecosystem. Both production traits and those suspected of being associated with ecological risk should be examined. In particular, comparison of hybrids which have experienced historical introgression events and those that are novel could be revealing.
4. Detailed genetic, molecular, physiological and behavioural studies should be undertaken with defined transgenic lines to examine the extent of indirect change which may have arisen as a consequence of transgene expression. Comparison



of several systems (*i.e.* different species, transgenes) which contain homologous or heterologous proteins expressed at different levels, times, and tissues should be examined.

5. The phenotypic characteristics of sterile transgenic fish should be examined for both their production characteristics and for their ability to interact and potentially impact the ecosystem directly throughout their lifetime.
6. Economic and sociological studies examining current data are needed to assess the true potential of genetic engineering (*vs.* alternative approaches) regarding the applicability of transgenesis to enhance food production efficiency in simulated field situations. Research developing genetic lines which utilize available and local plant-based protein meals and oils would be of great benefit.
7. The Trojan Gene Hypothesis, although theoretically possible, needs to be tested with rigorously-defined transgenic strains with significant growth enhancement. To date, examination of this hypothesis has been accomplished through modelling and a single poorly-defined ornamental model fish species. It is likely that this scenario is very case-specific.
8. Data indicate enhanced feeding behaviour of GH transgenic fish, but that this is accompanied by enhanced predation risk. The small amount of data available does not allow extrapolation of the lab-based information to the field. More complex apparatus and experiments are needed examining critical ecological variables (density, food availability and type, interactions with pathogens, etc.). Examining scenarios through multiple trophic levels would be of relevance to determine whether cascade effects are likely.
9. Research in nature is required to accurately determine the risks of releases of transgenic fish into nature. Since such releases are not allowed, estimates of fitness effects and ecological consequences must be obtained from confined laboratory studies. Research should be encouraged which examines the genetic and phenotypic characteristics of transgenic fish in laboratory scenarios, in mesocosms simulating to the greatest degree possible natural environments, and from surrogate systems (*e.g.* implantation of bioactive compounds which mimic transgenic phenotypes).
10. Research is required to understand the pleiotropic nature of transgenic phenotypes and their possible effects on the fitness of the transformed species. While currently it can be very difficult to extrapolate laboratory phenotypes to ecological consequences, laboratory studies are still needed to identify the basic phenotypes of the transformed organisms from which more complex experiments may be designed.
11. An additional long-term problem associated with risk assessments is the changing background genetics interacting with the transgene which will be selected over time. Observations have suggested that genetic background can affect the phenotype of transgenic fish, which indicates that, in nature, a transgene experiencing natural genetic variation within and among populations would likely shift phenotype in response to natural selection. This makes risk assessments a moving target. Thus, understanding the genomic control of transgene expression is critical.
12. New methods need to be developed for enhancing the efficacy of biological containment. Approaches include: 1) improvements of polyploidization procedures (*e.g.* efficacy of triploidy production through optimizing conditions



and/or production of tetraploid strains); 2) use of hybridization (directly if the hybrid is sterile, or in combination with triploidy if the diploid hybrid is lethal); 3) development of transgenic approaches to inducing sterilization or inviability; and 4) development of non-transgenic methods for inducing genetic changes allowing biological containment.

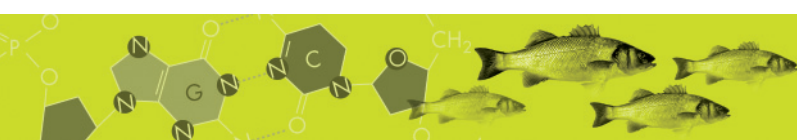
13. Research examining the economic feasibility of land-based aquaculture of genetically-enhanced strains could assist in decisions regarding the applicability of the technology for developed and developing countries.
14. Research on enhancing physical containment through improved engineering of culture facilities.

Recommendations

1. The current definition of the term “genetically modified organism” by EU should be reconsidered because it disregards organisms which are genetically modified by powerful genetic techniques, such as induced mutagenesis, is biased by the implicit assumption that genetic modification by natural mating and/or recombination is of less concern as opposed to transgenesis (invalid in the case of alien species), and does not distinguish between autotransgenesis with intraspecific gene transfer *vs.* allotransgenesis with interspecific gene transfer (see also annex).
2. Careful consideration should be paid as to whether there is a real safety problem posed to consumers by fish and crustaceans fed on diets containing transgenic plant ingredients. In particular, regulations about safe levels should not be arbitrarily fixed, but based on experimental evidence.
3. Evaluation of the possible promotional abuse of the term GMO-free (neither GMO *per se* nor GMO-fed) to improve the image of processed fish products that have no other truly enhanced quality or merit.

Suggested readings

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