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Review

Factors to consider before production and commercialization of aquatic genetically modified organisms: the case of transgenic salmon

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ABSTRACT

Many genetically modified plants have been developed, and four of them (soya, maize, cotton, and colza) representing more than 99% of commercial crops, are widely distributed, mainly in the United States and in America [ISAAA, 2006. Report on global status on biotech/ GM crops, Brief 35. International Service for the Acquisition of Agri-biotech Applications organization, US]. Yet all over the world policy is still in development in regard to authorization of modified plants and modified and/or cloned animals for food or feed and for their environmental release. The most advanced animal commercial projects concern various fish species, more easy to genetically transform, notably because conception and development take place in water and easy access to numerous eggs. A request for authorization to introduce genetically modified (GM) salmon onto the market has been presented to the Food and Drug Administration (FDA) of the US. In the interim, questions have been raised concerning the impacts of transgenic salmon, modified for productivity, on aquaculture, wildlife, ecosystems and on human health. Herein we review these scientific studies and sanitary, environmental, social and economic arguments. This paper analyses current gaps in the knowledge of the impacts of transgenic fish and proposes legislation orientations necessary for environmental and sanitary protection, should the marketing of animal genetically modified organisms (GMOs) be authorized.

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1. Introduction

Although no authorization for commercialization of genetically modified (GM) fish products for human consumption exists at present (beginning 2008) in North America and Europe, several genetically modified fish or shellfish (aquatic genetically modified organisms (GMOs)) are in development or have been said to be close to market for some years already (FAO, 2003). Among them, transgenic salmon is at the head of the list, and thus a review of this product may be useful to elucidate the numerous issues which should be considered within the authorization assessment process, including social, economic, public health and environmental concerns.

Some actors in aquaculture see in aquatic GMOs the possibility of improving the benefits of aquaculture (Melamed et al., 2002; Utter and Epifanio, 2002). This could occur through

increase of food conversion rates or the ability to assimilate vegetable feed, control of reproduction and sexual differentiation (e.g., one sex sometimes grows better than the other; mono-sex breeding may avoid losses in growth due to competition), acquisition of resistance to pathogens or parasites, improved tolerance to specific environmental conditions such as temperature, modification of behaviours such as aggressiveness, etc. Others suggest that aquatic GMOs will permit development of new organisms able to reduce harmful impacts of aquaculture on the environment, or produce molecules with therapeutic virtue or with capacity to detect pollution. Still, the reader is warned against hyperbolic claims with regard to advantages of aquatic GMOs and concrete developments towards theoretical projects. Modification of fish feed by genetic engineering or other means, is also an important research area today (Mente et al., 2003; Hevroy et al., 2004; Berge et al., 2005), as the production of aquafeeds (artificially compounded feeds for farmed finfish and crustaceans) has been widely recognized as one of the fastest expanding agricultural industries (FAO, 1997). It has also still to be carefully assessed.

In particular, environmental impacts should be studied in depth, as the release of genetically modified animals would, as for genetically modified plants, be irreversible. The introduction of new species in a given environment could be considered as similar to the introduction of a cocktail of new substances into a body: interactions and impacts are very complex and thus not subject to systematic predictability. Thus, as for toxicity, tests, and notably long-term tests, are necessary (Séralini, 2003; Séralini et al., 2007). These are conditions to maintain food quality for a high level of human health. Respect for protection against serious or irreparable harm is called for in Article 15 of the Declaration of Rio, even in the absence of scientific certitude.

The description, albeit complete, of a single function of an inserted gene cannot reveal unpredictable characteristics brought about by random insertions. In addition, given the knowledge we gain constantly of the complexity of genes, metabolic pathways and physiological functions, it seems reasonable to propose that risk evaluation should not be limited to the sole transgene but rather to the whole organism, understood as a wholly new organism, indeed one about which we may have relatively little or no knowledge.

The authorization process needs to be able to answer crucial questions, such as: (1) What are the risks associated with transgenic products released into the environment, in particular with respect to biodiversity and its serious decline? (2) Can an aquatic GMO, as an animal, food or food component, be shown to be innocuous, or free of health risks, including in the medium or long term of a lifespan? (3) What will be the social, cultural and economic impacts, in particular, on individuals and on small aquaculture companies around the world? As producers, will they be able to invest in this new technology? As consumers, will food choice become more limited?

The FDA received, a number of years ago (Yoon, 2000), a request for authorization to commercialize an Atlantic salmon, *Salmo salar*, engineered as ''AquAdvantage''. This genetic construction (opAFP-GHc2) used the Chinook salmon growth hormone (GH) gene combined with the ocean pout-antifreeze protein gene. It allows growth all year, permitting full growth to be attained in about half the time it takes for a normal salmon. According to the FDA website, ''most of the gene-based modifications of animals for food production fall under CVM (Center for Veterinary Medicine) regulation as new animal drugs'' (FDA, 2001). In December 2006, the company Aqua Bounty Technologies, Inc., claimed that ''the data and information submitted adequately supports the molecular characterization of the [gene] construct'' of their transgenic salmon, to the satisfaction of the FDA.

Although the decision-making process lacks transparency (Logar and Pollock, 2005) it will set precedents for future regulation of transgenic fish and other aquatic animals. Further, the information available concerning the regulatory process and the supporting documentation indicates the existence of difficulties in decision-making in regard to some innovations. It is clear, also, that various jurisdictions take markedly different approaches in this regard. However such approaches should be harmonized, as decisions made by one country may affect the others: if authorization processes are shorter or incomplete in a country, the result could impact on environmental and economic levels, including on the conditions of non-transgenic salmon. It is also obvious that different environments require different assessments. Environmental regulations will not work if nearby countries do not respect them (Jasanoff, 2005).

In this text we offer a policy input perspective based on current scientific understanding of transgenic salmon, rather than, for instance, a perspective grounded in one particular jurisdiction. This work is based on a bibliographic synthesis and personal experiences in the areas of molecular biology, GMO regulation, aquaculture and socio-economic risk analysis. The authors have familiarity with European legislation on GMOs (European Directives 2001/18 and 1829-1830/2003), Canadian legislative dispositions with regard to GMOs, European policy on chemical substances registration and evaluation (REACH, Regulation EC/1907/2006), as well as macrocosm approaches toward environmental impact assessments. This approach hopefully constitutes a useful entry point into a discussion of decision-making concerning aquatic GMOs and, more generally, genetically engineered organisms.

2. The salmon industry and its recent evolution

Salmon aquaculture appears, according to FAO, to be a growing source of food all around the world (FAO, 2004a,b), and yet it represents a source of pollution in particular via feed and fish wastes. Transgenic salmon may thus represent a threat to the future of the salmon industry and to wild salmon, through competition, worsening problems already observed.

2.1. A worldwide economy

The world production of farmed salmon rose from 484 thousand tons in 1985 to 1175 thousand tons in 2002 (GlobeFish Research Programme, 2003). Farmed Atlantic salmon constitutes more than 90% of the farmed salmon market, and more than 50% of the total global salmon market.

The most important salmon producers are Norway (460 thousand tons per year), Chile (260), United Kingdom (140) and Canada (110). These four countries furnish 91% of world production of farmed salmon, the largest portion of which is Atlantic salmon, *S. salar* (more than 1 million tons per year); a lesser portion is made up of two Pacific salmon species, *Oncorhynchus tshawytscha* (chinook salmon) and *Oncorhynchus kisutch* (coho salmon). There are in total at least five species of Pacific salmon belonging to the genus *Oncorhynchus*: chinook (*O. tshawytscha*), Chum (*O. keta*), Coho (*O. kisutch*), Pink (*O. gorbuscha*), and Sockeye (*O. nerka*).

International commerce in salmon reached 3.5 billion dollars in 2001, i.e., 7% of world trade in fishery and aquaculture products. Fresh salmon is now often the best seller in fish shops (Ofimer, 2005; FAO, 2003). For example, in France, in 2005, 20,399 tons of fresh salmon were bought by 36.5% of the households, amounting to an average consumption of 2 kg of fresh salmon, or 9% in volume of fish consumption (highest in Europe). Salmon is the second most consumed species, after tuna. This consumption is 90% made up of farmed salmon, representing 60% of all farmed fish (consumption of farmed fish versus wild: 14%) (Ofimer, 2005). If around 30 species have been genetically modified in the framework of laboratory research, AquAdvantage seems to be the main salmon for which the authorization process has been engaged, since several years now. Thus transgenic salmon was not (as of 2008) part of the salmon market.

2.2. Threat to wild salmon population

According to World Wildlife Fund and the Atlantic Salmon Federation (WWF and ASF, 2003), wild salmon stocks and biodiversity are in danger. Migrations for reproduction in rivers are increasingly disturbed by installations, pollution and the genetic drift due to escaped farmed salmon. In the outer Hardanger fjord on the west coast of Norway, 86% of the fish caught during 2003 were escaped farmed fish (WWF, 2005). The waste from salmon in marine cages or in fresh water hatcheries presents major problems, some of which are of the same type as those foreseeable with transgenic salmon.

Wild salmon traits show great genetic variability, a source of biodiversity which manifest in the form of many quite distinct populations in sea areas and in rivers. Farmed salmon, on the contrary, are raised and reproduced with an objective of genetic standardization, based on an aquacultural trait of interest such as better growth, less aggressiveness, or reduced resistance to pathology (Gausen and Moen, 1991). The crossing of wild populations with farmed salmon thus introduces new genetic combinations, the net effect of which may harm adaptation to the particular conditions of each geographic area, even of each river (Skaala, 1995). Genetic selection by stockbreeders in general produces salmon much less adapted for the search of food and reproduction in natural environments (DFO, 1999). Fitness reduction and the potential extinction of wild populations of Atlantic salmon are the result of interactions with escaped farm salmon, if only because the former are 48 times fewer in number than the latter (McGinnity et al., 2003). In wild salmon found in rivers in the Northwest of Ireland, two genetic markers showed a

crossing with Atlantic salmon escaped from marine cages anchored in a remote area (Clifford et al., 1998).

2.3. Fishing food dependence

Protein is of course, for a carnivorous fish such as salmon, an important part of the diet. This could make transgenic salmon a contestable choice in regard to the lack of food supply within the world. Lipid requirements are higher than for other marine species, around 25% of feed weight in adult food and even more in young stages. Although the conversion rate of this food into salmon flesh is high, sometimes attaining a figure near 1.5 kg food to obtain 1 kg salmon flesh (the rate depends on food quality, temperature, fish age, etc.) (Chamberlain, 1993), yet it should be recalled that millions of tons of small fish and crustaceans are transformed and through flour and oil enter the composition of food pellets destined to aquaculture. Most of the time, for each kilogram of flesh, salmon farmers use between 1.2 and 1.4 kg dry pellets, that is to say 4 or 5 kg of fresh fish and shellfish (Naylor et al., 2000).

In any case, there are increasing doubts regarding the long-term sustainability of farming systems based entirely upon these fishery resources (Naylor et al., 2000), in particular concerning the efficiency and ethics of feeding potentially food-grade fishery resources back to animals rather than feeding them directly to humans (Best, 1996; Hansen, 1996; Pimentel et al., 1996; Rees, 1997). It should be noted also that herring and sardines, important nutritional sources in salmon farming feed, are themselves excellent protein sources including sources of omega-3. This then poses a double set of socio-economic and ethical issues: the loss of food-grade fishery resources, and the transfer of these resources from the South (Africa and South America) towards the North (principal commercial outlet for salmon farming products).

Therefore, efforts will need to be placed on improving the use of fishery by-products (Alverson et al., 1994; New, 1996). The eventual success depends upon the further development and use of improved techniques in feed processing (Riaz, 1997; Watanabe and Kiron, 1997) and formulation, including the study of the potential use of specific feed additives such as feeding stimulants, free amino acids, feed enzymes, probiotics and immune-enhancers (Devresse et al., 1997; Feord, 1997; Hardy and Dong, 1997).

2.4. Sources of pollution

During breeding salmon show a high density, often equivalent to 30 kg/m of water. Increasingly, in a biological aquaculture setting, one tends to limit this density by half in order to lower the risks of detrimental consequences and to preserve the quality of fish. However, salmon, as a carnivorous fish still represents a particularly important source of nitrogen and phosphorus pollution. In Scotland, producing a ton of farmed salmon results in the release of about 100 kg of nitrogenous compounds into nearby waters (Roth, 2001). These forms of pollution, added to the uneaten particles and feces that fall on the bottom, deteriorate the benthic ecosystem not only under the netpens but also in a larger area around the fish farms. Elsewhere, this pollution can cause decreases in aquaculture productivity by promoting outbreaks of disease among the fish

(Naylor et al., 2000). In addition, as in aquaculture with other species, salmon farmers use a wide range of chemicals for prophylaxy or treatment as antibiotics; more than 51 such products are used around the world according to Bjorkland et al. (1991), as pesticides (algaecides, weedkillers, antifungics, antifouling paints) and as disinfecting agents and detergents.

In some cases, new technology has helped in a given socio-ecologic context. For example, in Puget Sound, on the west coast of the United States, one salmon farmer is using a giant, floating, semi-enclosed tub for breeding rather than the usual porous pens made of netting. The tub prevents fish wastes from polluting surrounding waters. Integrating the production of fish with other products, like seaweed and mussels that grow well in wastewater from intensive farms, can also help to reduce the nutrient and particulate loads. In Chile, some salmon are farmed with a red alga that removes nitrogen and phosphorous wastes from the cages. The effluent can also be used to produce a seaweed crop, offsetting the costs of creating the integrated farming system (Naylor et al., 2001a,b).

2.5. Transgenic salmon as a solution?

Existing programs of genetic selection of Atlantic salmon aim to improve salmon breeding performance (Gjoen and Bentsen, 1997), starting from populations selected generation after generation that will then transmit the characteristics of interest, such as speed of growth and late maturation, to the stockbreeders. Artificial maturation is also obtained by producing sterile triploid individuals or by techniques of continuous lighting. However, genetic recombination proceeds only according to natural genetic mechanisms governed by Mendelian laws of distribution of dominant and recessive genes to subsequent generations. Only the choice of the parents is artificial, i.e., different from those which would have taken place in a natural environment, but the genetic exchanges concern whole parts of the chromosomes.

Transgenesis is not simply a technological extension of such methods, but represents a revolution in that it makes it possible to modify a given part of the genome even somewhere where natural scission would not occur. This allows crossing the barriers of species or even of realms, a phenomenon that is still very little understood and surely generates novel physiological and metabolic conditions. It should be noted that positive results are more easily attained by transgenesis involving similar species, creating an ''all-salmon'' or an ''all-fish'' GMO as opposed to insertion, for example, of a mammalian gene in a fish (Devlin et al., 1994).

The biology of fish renders particularly simple the production of genetic modifications. Fecundation is external and development is carried out in an aqueous medium. Control of fecundation and recovery of eggs are relatively easy as it is possible to recover the mature ovocytes by ''stripping'', i.e., by the application of pressure on the latero-ventral sides. Fecundation will only start by mixing the ovocytes with the male milt obtained by the same technique and by adding water. Moreover, the high number (several hundreds to thousands) and the big size of the eggs (6–8 mm) facilitate microinjections of DNA. By comparison, the process in the case of transgenic bovine or sheep is much more difficult, not only because the eggs are much fewer and smaller, but also because these eggs must after microinjection and fecundation be reimplanted in the mother.

The first transgenic animal was a mouse in which a promoter of metallothionein was inserted to control the gene of the growth hormone in order to activate gigantism (Palmiter et al., 1982). After that, the first successes in aquaculture appeared rather quickly, in particular in Asia, with the transfer of human growth hormone into eggs of the common goldfish, to increase the growth rate of farmed fish (Zhu et al., 1985).

Many new transgenic aquatic species have been obtained since then, notably the Atlantic salmon in which the DNA fragment encoding the type III antifreeze protein was inserted (Shears et al., 1991) to allow a better development of aquaculture in Canadian zones where the temperature of water goes below 0, whereas the wild salmon cannot resist temperatures lower than 0.7 °C (Fletcher and Davies, 1991). Alternatively, in Atlantic salmon the introduction of an antifreeze protein promoter allows the stimulation of the growth hormone gene throughout the year and thus increased the speed of growth at least in the 1990s environmental conditions (Devlin et al., 1994).

Other possible uses of transgenesis have been mentioned in regard to the improvement of outputs and costs of salmon production, for example improvement of vegetarian food source for carnivorous salmon or improvement of food efficiency with a transgenic salmon (up to 20%). But until now, these ideas have not been developed as well as growth-enhanced transgenic salmon (Zhu, 1992). Will salmon become herbivorous in the future? Carnivorous fish do not easily adapt to vegetable-based food as special digestive enzymes are required (Cheng et al., 2004). Partial fish protein substitution is possible, notably by using extracted soybean protein concentrate (Krogdahl et al., 2003). However, vegetable lipids may change the flesh fatty acids profile, as mineral and trace element composition (Solberg, 2004); if composition and taste change dramatically, it could impact consumer acceptance. The direct consumption of vegetarian fish may also be a choice.

Other applications of transgenesis in fish have been proposed as well.

(1) *Fish as drug factories or as models to understand human pathologies*: Fish could be used as production units for molecules of therapeutic interest recoverable, for example, by extraction in sperm (Maclean et al., 2002). Fish may also be genetically modified to be used as a model of human pathologies (Grunwald and Eisen, 2002) or to understand the complexity and time course of genetic interactions, notably during various stages of development (Udvadia and Linney, 2003). For example, study of a zebrafish genetically modified to manifest a defective aortic valve development has made it possible to identify the role of an enzyme, UDP-glucose dehydrogenase, in the process of the embryonic development of this valve (Walsh and Stainier, 2001).

(2) *Fish as pollution detectors*: For example, the zebrafish *Danio rerio* has been genetically modified by inserting two DNA sequences in its genome, the first one a metallothionein promoter sensitive to presence in the environment of

certain heavy metals, and the second a reporter gene which produces the fluorescent protein of a jellyfish (GFP for Green Fluorescence Protein). These "bio-sensors" suggest a particularly promising method, in the context of environmental regulations, to evaluate eco-toxicological impacts of substances produced by human activity, including such chemicals as dioxins (Nerbert, 2002), estrogen-like substances (Chen and Lu, 1998) or polycyclic aromatic hydrocarbons (Amanumam et al., 2002).

(3) *Less allergenicity in food*: Further, transgenesis is proposed as a mean for removing allergenic substances in seafood. This type of approach could be of interest in salmon known to present certain allergenicities (De Martino et al., 1990) that can be at the origin of serious clinical symptoms, such as reactions of an asthmatic or anaphylactic nature. However, certain people allergic to some fish can possibly tolerate other species; this might represent a strategy more easy to implement.

3. Risks

The evaluation of the characteristics of terrestrial transgenic plants and animals continues to stimulate debate, including on the consequences of the process of transgenesis. With regard to transgenic fish, this evaluation is still only in the very early stages. Even if DNA analysis methods are well advanced, the protocols to detect transgenic salmon have yet to be developed, as is the case for toxicity tests (Zhang and Yang, 2004).

3.1. Genetic risk

3.1.1. The genetic modification by itself
Genetic engineering succeeds in overcoming natural limits in transplanting nuclei, manipulating the number of sets of chromosomes, or transferring DNA sequences. Aquatic GMO engineering may be considered as another step in this biology which circumvents natural cells and nuclear barriers by microinjection (Chourrout et al., 1986), electroporation (Inoue et al., 1990), sperm transfer (Muller et al., 1992), gold microparticule bombardment (Kolenikov et al., 1990), retroviral infection (Kurita et al., 2004), muscular injection (Tseng et al., 1995) or transposition (Raz et al., 1997), in order to introduce one foreign DNA fragment into the genome of a germinal cell. All these techniques represent an insertion by chance of one or several very precise genetic sequences in an unknown genome.

Thus, the novel trait of an aquatic GMO represents a qualitative change because most of the time it does not occur in natural populations of the parental species, or a quantitative one when the quantity of a natural substance is changed compared to the wild species. These changes affect a wide range of endpoints such as metabolic rates or endocrine controls, influencing a variety of functions such as reproduction, immune defence, nutrition, development and growth. In practice the most frequently observed phenotypic contribution derived from these changes is growth enhancement, and may also affect resistance or tolerance to threats such as disease, parasites or other adverse environmental conditions (see Section 2.5).

3.1.2. Random genetic insertion
Despite rapid advances in molecular biology since two decades, scientists do not have the capacity to control nor really understand the genome of living organisms. In particular, the risks of transgenesis arise from the lack of control over the number of sequences and sites of insertion, the rate of expression of the transgene, the complexity of interactions between the gene networks, the multiplicity of gene functions, epigenetics and the interactions with environment.

Here are three examples that underline the possible approximations of GM technology.

(1) In order to bypass the pituitary gland control of GH release during warm months, chinook GH genes were coupled to the antifreeze gene from Atlantic pout and microinjected into the fertilized eggs of coho salmon. Then, only 2–3% of the resulting fry exhibited expression of the gene. Nevertheless, after these transgenic fishes were mated to wild fish and the progeny with itself, 75% of the fry expressed the GH protein. Most of them then grew three to six times more rapidly and reached a market size (3–4 kg) a year earlier than their wild counterparts (Devlin et al., 1995). These discrepancies underline the lack of precision.

(2) At the University of Pukyong in Korea (Nam et al., 2001) microinjections were carried out, in loach eggs, with a genetic construction made up of the promoter of beta-actin combined to the growth hormone, the two elements coming from the loach itself. Nearly 7.5% of the transgenic fish had growth accelerated up to 35 times, and more than 65% of them transmit this character of gigantism to the following generation. However, with exactly the same protocol, i.e., the same construction and the same method of transgenesis, a considerable variability in the growth performance appeared with all the range from the "surprises" of a happy transfer to the many residual animals not presenting the anticipated properties (Zbikowska, 2003).

(3) Transgenic tilapia expressing tilapia GH cDNA under the control of human cytomegalovirus regulatory sequences, exhibited less food consumption and better food conversion. But these characteristics are in fact associated with many outcomes: synthesis retention, anabolic stimulation and average protein synthesis were higher, whereas some other metabolic states were different in juveniles, for instance, hepatic glucose. GH-tilapia juveniles show altered physiologic and metabolic conditions but from a commercial point of view the biologic characteristics were more efficient (Martínez et al., 2000). It seems probable that our understanding of the effects of gene insertion is less complete than usually represented.

Different families established from separate GH-transgenic salmons yield lines with unique growth characteristics suggesting important site-of-integration effects on transgene expression (Devlin et al., 2004a). The disadvantage of transgenesis is that, at present, the control of the transgene is unpredictable despite the known artificial promoter. It can thus not be expressed, or it may modify another gene by blocking it, by slowing it down, by stimulating it or by changing

its function. Thus, the transgene could make other genes function in an aberrant way.

Genomic rearrangements such as translocations and inversions occur randomly in nature. Although these rearrangements can be deleterious and reduce the organism's fitness, they probably present a lower level of risk, compared to transgenesis, perhaps because they fulfill some unknown roles circumvented when deliberate genomic rearrangements are induced through GM technology. In addition, novel regulatory control of gene expression is also possible by pleiotropic or epistatic effects of the introduced genetic construct. Sometimes, inserted DNA sequences do not act in the new host as they did in the donor organism, or alterations in one part of the genome caused surprising activity in other parts of the genome (Marx, 1988; Pursel et al., 1989). Novel regulation of gene expression has been for example linked to altered methylation of host regulatory elements (MacKenzie, 1990).

If some handbooks of biochemistry or molecular biology still retain and restate the axiom "one gene, one protein, one function", reality seems now to be much more complex, agreeing more with the theory of polygenic characters (Mather, 1979). In fact, the number of genes is smaller than the number of functions signifying that one gene plays several roles. Furthermore, interactions between genes are multiple and complex and generate novel functions; for example, most of the time a multitude of factors act synergetically to control one gene expression and this varies according to physiological and environmental conditions (Shrimpton and Robertson, 1988). Moreover, one gene can naturally exist in the form of several copies that "work" differently in different tissues or during different development stages (Séralini, 2004). In the rainbow trout, for example, it has been clearly demonstrated that the pituitary adenylate cyclase-activating polypeptide and growth hormone-releasing gene change their expression during development, notably through alternative splicing and variation in the gene copy number (Krueckl and Sherwood, 2001). Increasingly there is a debate around the very concept of the gene. Philipp A Sharp noted in his Nobel lecture (1993): "what exactly the gene is has become somewhat unclear" (Sapp, 2003). Indeed, our knowledge about hereditary mechanisms continues to evolve, including the fact that "the transfer of genes across the phylogenetic spectrum is now known to occur naturally" (Sapp, 2003). Today, genetically based knowledge is in a process of flux due to observations of an unanticipated "mind-boggling complexity" involving, for instance, overlapping genes, genes within genes, transcription (including overlapping transcripts, fused transcripts) converting many segments of genome (from either of the DNA strands) into multiple RNA ribbons of differing lengths and epigenetic inheritance (Pearson, 2006).

3.2. Health risk

Health risks may arise if the transgenic organism produces a new substance or an anticipated substance at higher concentration, compared to the non-transgenic equivalent species; this could therefore result in allergenic or toxic characteristics (Berkowitz, 1993). The GMO may also tolerate a new toxic compound or be sensitive to a pathogen (Séralini,

2000, 2004). Furthermore, in particular in the case of a hormonal substance, a complete change in many metabolic pathways could arise, rendering the aquatic GMO markedly different in chemical composition and thus contributing to unexpected risks which would need to be assessed (Malarkey, 2003).

It remains a problem that in some countries like USA and Canada, in contrast to the European Union and most countries that have signed and applied the Carthagena protocol, it is supposed in regulation that the whole GMO is equivalent to the corresponding wild species, necessitating no labelling nor mid- or long-term toxicity tests. This approach presumes that if only one new trait has been added, this will result in the production of only one new substance that does not change significantly the composition. For example the transgenic growth hormone salmon could be considered as a banal salmon that has only the particularity of producing more GH or a normal level of GH but all year round. This approach called "substantial equivalence" is risky because it is based on an oversimplified understanding of the complexities entailed in transgenic modification.

3.2.1. Allergy
As it was shown for a transgenic soybean containing a gene from Brazil nuts (Nordlee et al., 1996), the risk of allergenicity associated with the consumption of or contact with any GMO exists since it produces a new foreign protein that most often comes from another organism. Thus, genetic modification may result in making available immunoreactive structures which were previously hidden and/or nonaccessible to the antibodies. Biotechnological processes can also increase the level of expression and/or exposure to existing allergens, or even modify their allergenic potential (Wal, 2001). The impact of GM technology on the possible appearance of new allergens should therefore be studied in more depth before market commitment decisions are authorized (Wal, 1997).

Identification of new allergens may begin by comparing the sequence of a transgene with sequences already listed in data banks, that should present at least six amino acids common (Moneret-Vautrin, 2002). This method can be interesting solely if the molecule is directly produced by a particular transgene (not indirectly) and if the allergen is known. This approach *in silico* is thus very limited.

Thus, allergy tests made either in vitro with the serum of sensitive patients, or by cutaneous exposition of patients to the potential allergen, could bring additional security to the consumer. However, this approach is limited by the availability of the specific rapid and simple tests linked to a decision of public policy. Traceability of modified organisms and labelling is necessary for the consumer to recognize this kind of food.

3.2.2. Toxicity
Within major international organizations the concept of substantial equivalence has been presented as a useful part of a safety evaluation framework (now increasingly known as comparative safety assessment (Kok and Kuiper, 2003)), based on the idea that existing foods can serve as a basis for comparing the properties of GM foods with the appropriate counterpart (Kuiper et al., 2001). This approach is not

appropriate in evaluation of the safety of an organism modified for its metabolism like the described aquatic GMOs and should be changed, as suggested, for instance, by the Royal Society of Canada (Expert Panel, 2001), because nothing predicts that all the characteristics of transgenic salmon remain exactly equivalent to its non-transgenic counterpart (Blier et al., 2002). This is also considered true for all whole GMOs in a majority of countries, requesting mid- and long-term toxicity tests (Directive European Community 2001/18/EC), at least theoretically, until this Directive is scientifically applied as for pesticides and drugs.

Because of the random insertion and the genome complexity described previously, transgenesis can modify some biochemical pathways and/or physiological regulations in an aquatic GMO, which may then become, for example, a larger bio-accumulator of a pollutant that it tolerates (Kapuscinski and Hallerman, 1994). For instance polybrominated diphenyl ethers used as flame-retardants in several products of daily life, are now sometimes measured at levels averaging 1.46 ng/g wet weight in farmed Atlantic salmon in Chile (Montory and Barred, 2006). It was also often measured in human blood. Nothing guarantees that this rate could not increase in GH salmon that grows faster and have less time to eliminate this kind of toxic chemical.

Salmon dietary qualities are of interest in human nutrition and are associated with a positive image. Notably, proteins, polyunsaturated fatty acids (including the omega-3 group) (Sidhu, 2003), vitamin A and carotenoids (Rajasingh et al., 2006) content are high, especially in wild salmon. Evaluation should verify that these characteristics persist, especially in an animal that grows faster. Growth-enhanced transgenic salmon, compared to control fish, exhibited a 10% improvement in gross feed conversion efficiency, but body protein, dry matter, ash, lipid and energy were significantly lower relative to controls while moisture content was significantly higher (Cook et al., 2000). Similarly, essential amino acids and other elements were changed in other aquatic GMO species, such as GH-transgenic carps, showing that this should be taken into account systematically (Chatakondi et al., 1995).

Some examples of GM agricultural products show that unexpected effects should be prospected. When mice in gestation are fed with rations containing 14% of soy genetically modified to be glyphosate tolerant (the active ingredient of many weedkillers), modifications were observed in hepatic cells: irregularly shaped nuclei, a lowering in the concentrations of certain nucleolar and nucleoplasmic factors participating in the nucleic splicing process, as well as an abnormal accumulation of perichromatic granules (Malatesta et al., 2002a). (Transgenic salmon, in aquaculture, could also be fed with this GM soya.) This suggests a reduction of post-transcriptional processes (modification of RNA) and, thus, reduction of nucleic flow of acids from the core towards the cytoplasm. Elsewhere, the same GM food reduces zymogene granules and digestive enzyme secretions in mouse pancreatic cells (Malatesta et al., 2002b). A diet containing genetically modified soybean also showed some effects on mouse testis (Vecchio et al., 2004), maybe due to the traces of contained herbicide to which the soybean was tolerant. The immuno-labelling of some specific targets as the RNA Polymerase II showed a decrease notably in Sertoli cells of young GM-fed

mice. Furthermore a few cytological details were found modified in GM-fed mice of all ages: the number of perichromatin granules was higher, the nuclear pore density lower and the smooth endoplasmic reticulum of the Sertoli cells was enlarged (Vecchio et al., 2004). This could be explained by the fact that the herbicide Roundup containing glyphosate has been demonstrated to directly induce cellular toxicity in human embryonic and placental cells (Richard et al., 2005; Benachour et al., 2007) at doses that could be present in GM food or feed (dilutions 1/10,000). Furthermore, a commercialized GM maize called Bt MON863 has shown signs of hepatorenal toxicity after rat consumption for 90 days (Séralini et al., 2007).

In addition, even if some authors perceive a great difference between the growth hormones of fish and humans with, for example, 32% homology between red fish and humans (Mahmoud et al., 1996), these similar genetic sequences are important, especially when one thinks of the multiple physiological roles of growth hormone. It has been shown, for example, that the bovine growth hormone is able to activate, even with weak concentrations like 50 ng/ml, the synthesis of sexual steroids in the ovarian cells of sea trout (Singh and Thomas, 1993).

3.2.3. Horizontal gene transfers

Transgenic constructions may include marker genes to facilitate identification of the bacteria carrying the transgene. Generally, at present, it is a gene coding for resistance to an antibiotic which allows, in bacterial cultures containing antibiotic, to keep only the resistant colonies alive, i.e., bacteria having integrated the genetic construction in their genome. Thus the potential risk for horizontal gene transfer to the soil, bacteria, or organisms consuming the GMO should be studied.

Horizontal gene transfer occurs even more easily in an aquatic environment. For example, the ampicillin resistance from a transgenic *Escherichia coli* strain was found in another microorganism, Micrococcus (Popova et al., 2005). More generally, horizontal transfer from one species to another, even when both species are phylogenetically very different, seems to be a significant risk related to GMOs (Panoff et al., 2006). For example, transfer of resistance to streptomycin was demonstrated from a genetically modified plant, tobacco, to the bacterium Acinobacter (Kay et al., 2002). In the human digestive tract this kind of horizontal transfer could also be possible (Bertolla and Simonet, 1999; Kleter et al., 2005) and thus the removal of this kind of gene in commercialized GMOs has been proposed to avoid an increased risk of antibiotic resistant diseases (ACNFP, 1996). The proposal is still not applied in 2008.

3.3. Environmental risk

The presence of a high percentage of farmed fish among fish caught in the wild – up to nearly 30% – raises many questions about transgenic salmon (McGinnity et al., 2003). In the eventuality of an accidental escape, would transgenic salmon pose a threat to ecosystem equilibrium? Could they contribute to a reduction in biodiversity? Would it be possible for the transgene to be transmitted to wild salmon or other species?

What could be the possible consequences? Can transgenic salmon be reliably confined to prevent them from escaping? And in more general terms, if aquatic GMOs arrive on the market, each species will pose very different problems from an environmental point of view, because their biology and their reproductive cycles are very different; how can protection of the environment be assured?

3.3.1. Ecological knowledge

Guidance in assessing the capacity of an aquatic GMO to survive in nature and understanding which ecosystems it could access, will surely be furnished by a deep knowledge of the parental organism in natural environments and during stocking. One would look to the documentation of physical and chemical tolerances (temperature, salinity, pH, dissolved oxygen, etc.) and biological factors needed by the species (habitat, predators, pathogens, nutrient requirements).

However, this background is not sufficient and should be strengthened with other information. Thus particular attention should be given, notably, to cases where survival and persistence occurred contrary to expectations. From time to time, it is observed in fish ponds that birds catch fish, transport them some distance, but lose them before consuming them; in this way, new fish species or disease can be transferred in or out of fish ponds, or from one river to another. Another unexpected example could be cited: a Canadian salmon hatchery had made the assumption that the juvenile stages would survive only in the waters around the farm and simply flushed into Lake Superior more than 20,000 juveniles. Twenty-four years later, however, the pink salmon (*Oncorhynchus gorbuscha*) population, previously inexistent in this area, exploded in the Lower Great Lakes (Emery, 1981).

Although the zone of tolerance of a given aquatic GMO to physical and chemical factors must be considered to evaluate its potential for colonizing accessible ecosystems, this information is nonetheless probably insufficient. Firstly, assessment of the tolerance to individual parameters is not problematic, but assessment becomes more complex with the combination of different factors. Secondly, in certain conditions, the organism could survive long enough to pass through an ecosystem that has bad conditions, to finally reach another habitat where the animal could then persist and reproduce. It was observed that Tilapia persist several months in a temperate ecosystem until temperature declines in winter, this delay allowing the tropical fish to "prospect" for new territories. In coastal wetlands in south-eastern Mississippi, the presence of an aquaculture downstream thermal area unexpectedly provides a refuge for continued survival of released Tilapia (Peterson et al., 2005).

3.3.2. Biodiversity considerations

Environmentally safe research and commercial production is particularly important to protect biodiversity (Convention on Biological Diversity, 2006). Other aquatic organisms such as molluscs and crustaceans could join salmon in being genetically modified with the aim of market commercialization and introduction into the environment. This could, however, affect biodiversity worldwide, and aquatic biodiversity has already suffered dramatic declines (IUCN, 2004). For example, since 1970 a dramatic decrease of freshwater biodiversity has been observed, with more than a 50% decline in species populations (WWF, 2006a), and in oceans 27% of fish fauna is endangered, threatened or of special concern (Hugues and Noss, 1992). Yet until now we have identified only a fraction of the earth's biological diversity and thus have just a rudimentary understanding of how biological, geophysical, and geochemical processes interact to contribute to human and ecosystem well-being (WWF, 2006b). Therefore, protection of this natural diversity at genetic, species and population levels is of paramount importance. We have only begun to consider the untold costs of lost biodiversity and the potential gains of biodiversity maintained (Pimentel et al., 2004). Commercial fishing and its growing technologies already exert pressure on biodiversity, and GM biotechnology could further their negative effects.

3.3.3. Three possible environmental scenarios

To predict the environmental impact of escaped transgenic salmon is a particularly difficult exercise. As is known since Darwin, one genetic trait is able to change a whole population, and as is known since Möbius, many interactions exist between organisms in a given ecosystem. Who could have predicted the excellent adaptation of the tropical alga *Caulerpa taxifolia* to the coasts of the North of the Mediterranean Sea except in some areas around Corsica (Boudouresque and Verlaque, 2002)?

Transgenic fish modified for fast growth can fairly quickly, as new arrivals in an area, become strong competitors in search for food (Devlin et al., 1999), habitat and/or reproduction (Johnsson and Björnsson, 2001), in predator avoidance (Dunham, 1995), and this even if sterile (Masaru et al., 1993). It has been observed, for example, that transgenic coho salmon *O. kisutch* were more willing to take risks when feeding (Sundström et al., 2003). In a longer time frame, the heterogeneity of the wild populations could also be seriously reduced by a "genetic flow" resulting from the escaped transgenic salmon crossing with the wild populations (Kapuscinski and Brister, 2001; McGinnity et al., 2003). However, many traits that appear to confer an advantage in the short-term could have long-term costs that make them overall detrimental. For example, domesticated trout, that grows faster but takes more risk during feeding, do not tend to survive when predators are abundant, compared to wild trout (Biro et al., 2004).

The potential effect of such genetic flow has been explored using as laboratory and field models tropical species smaller than salmon and reproducing more easily and faster, such as Medaka and zebrafish. In addition, simulations were conducted taking into account factors such as the weight of the genetic traits (ecological advantages and disadvantages) and their Mendelian transmission. Results show that "the invasion of transgenes" in wild populations is very probable, even were only some individuals to escape into the natural environment (Hedrick, 2001; Muir and Howard, 1999). Further, it was shown that each transgenic fish is a particular case, and that some traits could drive the long-term effect of the genetic invasion. For example, the consumption of more oxygen could be unfavourable to the development of a transgenic population (Stevens et al., 1998), whereas a higher size of sterile males or a lower rate of viability of the offspring could render both

wild and transgenic populations extinct (Andersson, 1994; Howard et al., 2004).

Thus, if transgenic fish are introduced into a wild population, modeling and experimental studies give raise to three hypothetical environmental scenarios after several generations of cohabitation (Hedrick, 2001; Muir and Howard, 1999, 2002):

- *Elimination*: After some generations, the transgene associated with a major disadvantage is gradually eliminated from the population and the fish population is composed finally of the wild genotype. The impact on the wild population can however be more or less important during the few generations necessary to ''purge'' the transgene.
- *Invasion*: Because it is associated with a major advantage, like an earlier sexual maturity, the transgene is progressively propagated by regular crossings with the wild type to finally be present in all the genomes; the wild genotype thus disappears completely.
- *''Trojan gene''*: The gene is propagated in the wild population during several generations thanks, for example, to a reproductive advantage, but because associated characters, such as low rates of larval survival, are nonviable, the tendency is toward extinction of the whole population. It was estimated that if 60 transgenic salmon were disseminated among 60,000 wild salmon, the natural population would be decimated in 40 generations (Howard et al., 2004).

3.3.4. Physical and biological confinement

The great mobility of fish and their reproductive strategy, built on abundant eggs deposited in rivers, make the environmental risks much greater in the case of these animals, compared with terrestrial vertebrates.

Cages lose fish by direct predation or because the installations are damaged by predators such as fish and birds, and also sometimes by poachers. Bad weather conditions – swells and storms, currently increasing with climate change – are also able to degrade a marine farm. And in everyday operations, handling and transferring fish, for example, with boats and other mechanical devices, losses can occur. Sometimes, even if only once, as many as several hundreds of thousands of salmon escaped from nets (Hallerman and Kapuscinski, 1992). Strict confinement is almost impossible.

Land-based systems of breeding, confined within physical structures isolated from natural waterways by filtration apparatus and other forms of water treatment, give a more robust guarantee of effective confinement. However, even so, risks are not totally absent, in particular when a hatchery is on the site, because the number and the small size of eggs and fry make it very difficult to assure confinement. Dissemination in the environment is possible, for example, on water droplets transported on clothes. Discipline in handling and transport is of course able to improve biosafety but will not overcome human error, in particular if the application of protocols and work conditions are not strictly regulated and supervised.

Control of the reproductive capacity of transgenic fish is one solution advanced to mitigate the environmental risk. Triploidization is the tentative sterilization technique most often employed. By a chemical or physical stress, it prevents the normal separation of the chromosomes at the time of

meiosis leading to sets of 3*n* chromosomes. The process appears sometimes in nature, and these laboratory procedures are currently used in aquaculture, notably to enhance maturation (Lincoln and Scott, 1984) and to increase growth rates (Seeb et al., 1993).

Alternatively, more sophisticated approaches can be considered. For example, a suitable maternal species could be subjected to gene transfer and perpetuated as an all-female transgenic line, whose eggs are fertilized with cryopreserved sperm of a compatible paternal species in order to generate sterile transgenic hybrids. After that, to yield 100% transgenic offspring, the transgene should be fixed in the maternal line in a homozygous state through at least three generations (Colombo et al., 1998).

There is need to validate the success rates claimed for such procedures of sterilization or containment. Nevertheless it can never be 100% in biology. Moreover, sterile transgenic salmon, even unable to reproduce, can interfere in the reproduction of wild salmon, for example, by competition for the food resources (Muir et al., 2001).

3.4. Socio-economic risk

Different socio-economic frames induce different risks. One link with the socio-economic risks is that if salmon is sterile, fish farmers would be totally dependant on companies commercializing the GMOs. This will probably drive out of business many family fish farms all over the world in favour of a few big companies. Moreover, the rapid growth of commercial fish farming over the past decade has led to sharp decreases in salmon prices. Once commercially available, transgenic salmon could flood the market, driving down the price of farmed salmon even further. Falling prices could put some farmers out of business while forcing others to accept the new technology – willingly or unwillingly – for fear of losing out economically.

In general, the cultivation of GMOs is currently associated solely with large-scale production. In most cases, this production does not benefit countries where the greatest food needs prevail. Rather than bringing food products and food diversity to local communities, GMO technology has on the contrary tended to bring the fruit of its production to world markets and this patented technology has not been financially accessible to small-scale farmers whose focus is rather farming as a ready source of subsistence. Similar scenarios are foreseeable for aquatic GMOs. Elsewhere, it could be questioned if transgenic pollution sprayed in aquatic environments could perturb the supply in wild salmon for ''traditional'' salmon farming. Conflicts could appear similar as between transgenic agriculture farmers and organic farmers (Conner et al., 2003).

As stated before (see Sections 2.2 and 2.3), the heavy exploitation (and often, overexploitation) of natural marine resources (Gill, 1997) is also a factor which may deeply influence the salmon industry in the future. Better food production efficiency will be a challenge for this industry, and at least three technological strategies will be used to answer the challenge: use of aquafeed and genetic modification both will entail more dependence and more investment and thus, similarly to industrial agriculture, will demand intensification

of production, or more pragmatically, the choice of a less carnivorous species and less sustainable development.

Transgenesis is an additional stage in the impoverishment of the genetic pool of salmon, whereas it is well known by farmers that genetic diversity is often the best weapon against pathologies. For example, in Europe, the oyster *Ostrea edulis* was contaminated in the 1920s by two parasites *Marteilia refringens* (Marteil, 1971) and *Bonamia ostreae* (Comps, 1985a); the oyster *Crassostrea angulata* became then the solution to save the oyster economy. In the 1970s, *Crassostrea angulata* was also weakened by a viral epizooty that killed more than 90% of this too homogeneous production (Comps, 1985b), and again another species, *Crassostrea gigas*, was the solution to help the oyster farmers to survive after a major social crisis.

Last but not least, appropriation of ownership over life forms, through patents, is contributing to widespread debate which should integrate of course ethical considerations. Patents on life forms promote the ''artificialization'' of ecosystems and the possibility of establishing monopoly control over parts of it. Moreover, such biotechnologies support a two-speed aquaculture, which in the long run will be unfavourable to small-scale farms and the poor countries. Whereas these farmers should continue polyculture, with the objective of maintaining their self-sufficiency, the adoption of aquatic GMOs would push these farmers towards dependency on multinationals, as observed with agriculture farmers (Friends of Earth, 2007). This could be especially true if sterilization was systematically adopted as one solution to environmental threats, a scenario which some companies and countries have adopted in agriculture, with the sterile seeds known as ''terminator'' or the genetic use restriction technologies (GURTS).

4. Some propositions for risk assessment in the case of a transgenic fish

While a faster-growing salmon is one of the first transgenic fish, the biotech industry is seeking to introduce many others GM animals to market. Scientists worldwide have altered the genes of at least 30 other aquatic species, including flounder, carp, lobster, and shrimp for both scientific study and commercial production. Terrestrial transgenic animals in farms are also developed, but not commercialized such as pigs which produce meat with less fat, chickens resistant to bacterial infections, and cows that can grow faster on less feed. It is obviously important, therefore, that the decision to approve or not approve this first transgenic animal be well done.

The proposals outlined below aim to take into account knowledge which exists about transgenic aquatic organisms, while recognizing zones of ignorance. Further, policy work has already resulted in some general orientation in this area, including expert opinion sought by FAO/WHO (FAO/WHO Expert Consultation, 2003), legislative initiatives in Europe (starting with Directive 2001/18/EC), ongoing reflection in and international collaboration from Canada, and at least one scientific initiative aimed at identifying risk components to be evaluated before adoption of aquatic transgenic organism production (Scientists' Working Group on Biosafety, 1998).

It has been well recognized that regulation of aquatic GMOs should be based on principles and criteria agreed upon by scientists and in international political arenas. We may mention, for example, early initiation of citizen participation and consultation, and principles of sustainable development, substitution, duty of care, and precaution. International agencies (such as OECD, FAO/WHO, Convention on Biological Diversity) and important national authorities (such as the EPA in the US) have adopted these principles, thus leading the way to establish them as guideposts to conceive an ambitious process of rigorous evaluation and risk management. In these matters, it seems clear that acceptance by the public will be facilitated by strong guarantees of safety with regard to health and the environment.

4.1. Principles

It is generally recognized in Western countries that science and technology (S&T) have contributed strongly to a general increase in standards of living and health. However, we now understand more clearly how these ''advances'' may be accompanied by previously uncalculated environmental and socio-economic costs. Thus, an increasing challenge faces policy makers: to succeed in deriving optimal benefit from S&T while concurrently supporting precautionary approaches in regard to public health, environment and social equity, which are the basis for sustainable development.

It has been proposed that the general principles of evaluation of transgenic crops could be applied to GM animals (FAO/WHO Expert Consultation, 2003). Some elements of evaluation will, however, necessarily be specific to animals and, even more, to aquatic animals. Furthermore, this should be developed in continuity and in coherence with rigorous and evolving codes and guidelines, such as: (1) state of the art standards of scientific evaluation and research on GMOs, (2) national and international legislation and regulations on the production, the transport and the marketing of food resulting from GMOs, (3) the Convention on biological diversity, and (4) FAO's Code of Conduct of the activities of fishing and aquaculture. Elsewhere, fundamental and traditional principles concerning environmental protection rights should be used such as the Amerindian precept according to which ''we did not inherit the ground from our ancestors, we borrowed it from our children''; or the International Declaration of Stockholm in 1972 to promote ''the right to an environment of quality''; and the principle of precaution as enunciated in Principle 15 of the Declaration of Rio in 1992.

The notion of alternatives to new technologies is now a principle increasingly adopted in legislations, for examples in the Stockholm Convention on Persistent Organic Pollutants or in the European regulation ''REACH'' concerning the Registration, Evaluation, Authorization and Restriction of Chemicals (EC/1907/2006). This approach also theoretically promotes the consideration of the future risks entailed in particular options with regard to fulfilling an identified need (GMO-ERA, 2004).

The ''Polluter Pays'' principle has been also proposed, and it charges the expenditure relating to possible pollution to the responsible parties, in particular through payment of ecological taxes. In our example, these green taxes could be paid by companies that engineer the transgenic salmon as salmon

farmers who use it. This principle, recommended by the OECD, is also put forward by the European Council. Further, there is need to explore other avenues through which the civil (including financial) responsibility of innovators and developers is ensured. Another possibility is to adopt an international ban on techniques considered too risky, too difficult or impossible to control, especially from an environmental or socio-economic perspective.

Each aquatic GMO should be considered unique, and analysis of the health and environmental risks should be pursued on a case-by-case basis. A transgenic salmon (a growth hormone-modified salmon, for example) may not be identical to another, even if the two salmons are elaborated within the same company and on the same day, due to differential transgene insertion for instance.

In the event that transgenic salmons were to be considered equivalent to their wild counterparts, a reduced amount of information would be produced concerning the transgenic populations. In this eventuality, a legally binding "duty of care" becomes all the more important. Giving this duty legal status would codify existing voluntary commitments of the aquaculture biotechnology industry, thus ensuring that producers, distributors and transformers of transgenic salmon are responsible for their products and assuring both reassurance and recourse for citizens and consumers. To ensure such legal duty we must consider the necessity to establish a superfund like the one established by EPA.

Furthermore, downstream users and the public could have access to all information relevant to environment and health with regard to GMOs, thus allowing them to understand and intervene in issues of security standards. Sufficient information could be given to enable retailers and consumers to find out about transgenic aquaculture products, notably by systematic labelling of all products which goes with traceability, as a tool for choice, and also as an insurance for all food and feed producers in case of any problem.

It is also essential to call for a strict counter-expertise embedded in public policies, so as to counter-balance information provided by industry. Independent contribution by experts and by the public as well, should be planned and implemented at all the stages of the regulation elaboration, as called for by FAO/WHO expert consultants (FAO/WHO, 2003). In addition, if new scientific information emerges after authorization has been accorded, showing the need for wider precautionary measures or even prohibition of an aquatic GMO, then procedures should allow for modified and emergency measures. A clear separation of responsibilities between companies and researchers who propose GMOs, and policy makers setting protection rules and risk assessment guidelines, constitutes a key element of public policy quality. International indications of growing concern show that the public wants to be involved in developing biosafety regulations (McLean, 2005).

To resume, important facets to be considered should include: (1) measures and evaluations of health, environmental and socio-economic risks, (2) determination of conditions to be respected in the stages of production, handling and transport. Further, there should be clear and well-defined procedures to be followed for, (3) authorization of production, distribution and marketing of GMOs and especially aquatic

GMOs, (4) control mechanisms which should be put into place, with guarantees as to transparency of process and results. Also, there should be (5) early and complete information-sharing and effective consultation on the relevance of the project on all its aspects with citizens, including consumers, and (6) ongoing information availability, for instance through traceability and labelling.

4.2. Identification of the GMO

The description of the GMO should be delivered following different headings. Firstly there should be a general description of the host including its taxonomy and variability, the genetic trait to be modified, the method of genetic transformation and the DNA source. Under a second heading, one should find a detailed genetic description of the introduced material sequenced after transgenesis, real transcripts and protein encoded, with the knowledge of surrounding genes and potential interactions. Thirdly one should find a description of the variations in the functions and the expression of the transgene, especially those not envisaged initially by the construction. The knowledge of the number of copies and places of insertion of the transgene(s) would reduce considerably the doubt around the identity of the new organism, and enhance the quality of traceability. Certain molecular strategies, called "directed" transgenesis, are already able to improve this control and should be preferred (Tronche et al., 2002).

4.3. Assessment of toxicity

A complete evaluation of the toxicity and the pathogenicity of the whole aquatic GMO and its genetic components is necessary. The development and validation of new profiling methods such as DNA microarray technology (Von Schalburg et al., 2005), proteomics, and metabolomics for the identification and characterization of unintended effects, which may occur as a result of the genetic modification, promise to furnish tools which will help to draw a good basic molecular profile. However, the basic strategy resides in more classic allergenicity and toxicological assessments, albeit with modifications in the test sets required and in technical elements of the protocols.

4.3.1. Evaluation of the allergenicity
The degree of allergenicity must be evaluated so as to inform sensitive individuals (FAO, 2001). Ideally, an evaluation by comparison with sequences of known allergens to find homologies of at least six similarly aligned amino acids (Gendel, 1998) and by pepsin degradation testing (because an easily degraded protein is less likely to be of risk), combined to in vitro tests of reactivity of immunoglobulin E of blood (Moneret-Vautrin, 2002) and/or cutaneous tests on humans. This could provide a better level of safety to inform consumers, if there is clear separation of channels of production, traceability and labelling.

4.3.2. Evaluation of toxicity
Given the importance of ensuring the safety of new foods, an aquatic GMO should be examined as a new organism. It is not

simply ''an animal drug'' even if US FDA is actually evaluating AquAdvantage transgenic salmon in this framework. Firstly, a complete chemical analysis of the various nutrient groups and also of the pollutants potentially accumulated in the animal is necessary for health considerations, especially when no particular advantage for the consumer can justify any added risk. Secondly, it would be inadequate to focus solely on the protein expected to be produced from a transgene (for instance, from growth hormone insertion), because this would not consider all the modifications or unexpected results, due to random insertion, generated by the transgene. Therefore toxicological tests should not simply estimate if the hormone is overexpressed regarding our knowledge about sequence homologies, digestive half-life or human blood rate, but rather check all the possible effects of an unknown product, without any assumption. Thirdly, sub-chronic and chronic series of tests of toxicity are necessary to identify any risk, including unexpected ones. Indeed ''substantial equivalence'' is a concept used in evaluations of GMOs intended for consumption, to distinguish organisms needing more complete evaluation from those needing less. This practical approach is meant to determine the safety of new food by comparison with similar, traditional food (OECD, 1993), and it has also been recommended in the case of aquatic organisms resulting from biotechnologies (OECD, 1994). However, it can indicate, in particular, acute risks of toxicity, but as a criterion or test is not very powerful for prediction of risk of chronic or sub-chronic toxicity in which large set of endpoints are checked as reprotoxicity, immunotoxicity, teratogenicity, genotoxicity, hepatotoxicity or unspecific toxicities.

4.3.3. *Modification of the OECD chronic oral toxicity test 452*
The studies of chronic oral toxicity, carried out to evaluate the cumulative toxicity by prolonged and repeated exposure to a drug over a minimum period of 1 year, usually follow the OECD Directive no. 452 (OECD, 1981). This approach is not adopted in the international regulation of GM crops, but it could be used as a basis to assess the long-term toxicity of an aquatic GMO such as transgenic salmon, using a 2-year minimum duration of tests on laboratory mammals to better approximate consumption realities. When extrapolating to humans, particular attention should be given to potential special sensitivities of certain populations such as pregnant women and children. The implementation of these guidelines would generate data helpful to identify the majority of chronic effects and to determine even non-linear dose-response or age, time- and sex-related relationships. Ideally, the protocol should allow for detection of all the general toxicities including endocrinological, neurological, physiological, biochemical, and haematological effects and exposure-related morphological effects.

In chronic toxicity studies proposed here, endocrine effects may not follow a linear dose-response. Sex steroids and reproductive functions require particular attention. Therefore, at least three dose levels should be used after a choice in preliminary experiments. The highest dose level should correspond in nutrition to the maximum acceptable to a physiological point of view and should always be applied within a balanced diet. The route of administration would be, of course, oral.

Careful clinical examinations should be performed at least on a weekly basis during chronic tests for mammalian or animal consumption. They should include neurological and ocular changes as well as mortality and morbidity. Body weight and food and water intake should be recorded weekly, whereas detailed haematological examination should be performed at 3 months the first time, and at 6-month intervals thereafter. At the same intervals, urine samples should be collected for analysis to determine appearance, protein, glucose, ions, ketones, occult blood and microscopy of sediment; and clinical chemistry measurements in plasma should determine total protein concentration, albumin concentration, liver function tests (such as alkaline phosphatase, glutamic oxalacetic transaminase and gamma glutamyl transpeptidase), carbohydrate metabolism (such as fasting blood glucose), and kidney function tests (such as blood urea nitrogen) as well as sex steroids. Histopathological examination, macroscopic as well as microscopic, is often the cornerstone of the chronic toxicity study. These aspects should therefore receive all necessary attention and should be described and reported in detail, including diagnosis. A well-performed gross necropsy may provide optimal information for microscopic examination and may in certain cases facilitate more restrictive microscopic examination. All organs and tissues should be preserved for microscopic examination. This usually concerns brain, pituitary, thyroid, thymus, lungs, heart, aorta, salivary glands, liver, spleen, kidneys, adrenals, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, uterus, urinary bladder, lymph nodes, pancreas, gonads, accessory genital organs, female mammary gland, skin, musculature, peripheral nerve, spinal cord, sternum with bone marrow and femur (including joint) and eyes. All grossly visible tumours and other lesions should be examined microscopically. In addition, microscopic examinations should be conducted of all preserved organs and tissues, with complete description of all lesions found; and the organs or tissues showing abnormalities caused, or possibly caused, by the aquatic GMO food should also be examined in the lower dose groups. Because transgenic animals could substitute without experience the basis of the food for the whole population, these parameters should be inspired by what is presently done for drug assessments. A modified metabolism in a GMO could make it more sensitive to diseases provoking in turn an unsafe consumption.

The test report must include all information necessary to provide a complete and accurate description of the test procedures and an evaluation of the results. It should contain a summary of the data, an analysis of the data, and a statement of the conclusions drawn from the analysis. The summary must highlight data or observations and any deviations from control data which may be indicative of toxic effects. In addition a 4–8 page scientific paper summarizing the materials and methods, the results and the interpretation should be provided. The crude data should be available for the scientific community.

In addition to the studies of animal toxicity described above, other special studies can be required to obtain information on specific effects of the aquatic GMO linked to the transformation. Flexibility for a case-by-case adaptation should be integrated into the regulation, in order to not miss any toxicity.

4.4. Assessment of environmental risks

Knowing that "ecological knowledge about potential environmental effects of transgenic organisms is crucial for understanding and avoiding these types of risks" (Ecological Society of America position paper: Snow et al., 2005) and that the environmental impacts of transgenic salmon can be irrevocable (Muir and Howard, 1999), a strategy of prevention is necessary. This should be based primarily on a set of impact studies, pursued under at least five headings: (1) analysis of the state of a site and its environment, (2) analysis of GMO's direct and indirect effects on the environment, (3) reasons for which a project is proposed, analysed with respect to alternatives, (4) analysis of provisions envisioned to eliminate or reduce environmental damage, and (5) critical analysis of impact measures and methods proposed.

4.4.1. Biological and environmental knowledge

Detailed phenotypic descriptions including, in particular, environmental knowledge of the wild counterpart and results of experiments in confined artificial ecosystems will contribute to the evaluation of the environmental risks. However, if aquatic GMO farming is eventually authorized, a field monitoring program should also be planned to collect further data in order to alert and/or improve biosafety management, as it is done by the "Resources Agency" of California, which collects data year round on the distribution and relative abundance of all races of juvenile chinook salmon using the Delta and lower Sacramento River (California Department of Fish and Game, 2005).

The European Community improved its environmental protection policy, but there is still room for progress. For example, "the scenario approach" which models expositions is based most of the time on a few accurate eco-toxicological tests (i.e., European Biocides Directive EC/98/8). Therefore, genetic flow should not be estimated only by experiments with other species (for instance, tropical fish to assess transgenic salmon) coupled to modelization, but also by macrocosm case studies, in order to assess the transgene stability, the genetic flow towards related wild species, and the effectiveness of fertility or sterility.

4.4.2. Macrocosm approach

In addition, the macrocosm approach could, keeping in mind that any introduction of a new species into an ecosystem can in cascade destabilize its equilibrium, help to investigate other ecological endpoints as whether the voracity of GH-enhanced salmon could be a threat to other fish species (Devlin et al., 2004b). Ideally, one would study each new transgenic line within macrocosms, which are artificial ecosystems which mimic the natural environment, while remaining completely enclosed, allowing testing of various cyclic phases and ecological conditions. These systems should closely resemble real environmental conditions and be sufficiently replicable to be statistically processed. This means that they should contain all the likely species with which the salmon could interact directly or indirectly in a natural environment, as also the main physico-chemical conditions met in the field such as water currents, presence of specific habitats with plants and gravels, etc. All the pertinent questions should be asked for each new species and its specific environment, and the experiment designed to answer them. For example, the most important questions – but not the only ones – that should be asked in the case of GH-transgenic salmon are: what are its tolerances (which ecosystems will it be able to colonize)? Will it be a strong competitor for food with wild salmon and also, for example, with sea trout (Bakke et al., 1999)? Will a genetic flow occur with the wild? In order to answer these questions, experiments should be then split into several sub-experiments to test the different life stages of the salmon. There are a multitude of biological parameters which will have to be studied as food behaviours, aggressiveness, capacities of escape from containment structures and of migration, reproduction aptitudes, survival rates, tolerance to physiological stresses like containment and changes in temperature and oxygen, etc. Moreover, the approach must be multidisciplinary associating molecular and physiological aspects with ecological ones (Hodgson and Sugden, 1988; Tiedje et al., 1989).

4.4.3. Sterilization

Sterilization of transgenic fish appears to be a biosafety measure impossible to circumvent (Kapuscinski and Hallerman, 1990; Seeb and Miller, 1990). Although sterilization would greatly reduce the genetic pressure of transgenic salmon on wild stocks, it cannot guarantee 100% efficiency (CEQ, 2001), nor can it preclude competition for tropic resources, habitat and reproduction. Even these sterilization methods are relatively easy and sometimes allow rates close to 100%; for example, for triploidization in salmon, the results vary according to the species and the techniques employed. Thus the reliability of the method of sterilization must be measured and considered in the evaluation of the risk on case-by-case, in particular knowing that the regular release of small quantities of fish can have an impact as pernicious as a very spectacular massive release (Maclean and Laight, 2000).

4.5. Implementation and management

4.5.1. Biotechnological revolution

It seems that caution and circumspection should be applied to our understanding of the claims in regard to an imminent "biotechnological revolution" (Nightingale and Martin, 2004). A variety of motivations underlie discourses on the so-called revolution. In order to generate investments for research and development, the actors of biotechnological innovation need to raise hopes and interest in the future benefits both therapeutic and economic. Development and marketing of a biotechnology-derived food or drug is a long process, with many distinct stages characteristic of the scientific, technological, financial, commercial and marketing aspects involved, to name only those. The process is therefore characterized by significant risks and often complex forms of collaboration which, varying according to the sector, may involve years, even decades, before innovations bear results from the market (Benneworth, 2003; Teitelman, 1989; Pisano, 2006). Lastly, non-governmental organizations report disappointing results and consequences from transgenic corn crops, such as contamination of "biological" crops, weaker outputs than in traditional (non-GM) agriculture and questionable protection from

insect pests against which GMOs were supposed to be protected (Friends of Earth, 2007). Transgenic salmon regulation should not follow this long and uncertain route of criticisms. It should be comprehensive in the first instance.

4.5.2. Participative decision-making

Variations in national treatments of science and technology are influenced by particular interests. They influence in turn the understanding and degree of acceptance which citizens show with regard to new knowledge and practices. Evaluation and orientation toward new and better practices is thus most wisely approached in collaboration with the broadest range of interested parties, thus ensuring a more complete understanding of the issues. Effective citizen participation should start at the earliest possible point in the process, starting with relevance of the project (Vandelac, 2006) and continuing on (FAO/WHO Expert Consultation, 2003). In Canada, for instance, numerous parliamentary committee hearings and government reports describe a complex set of issues and interests which characterize current Canadian aquaculture practices. This contributes to constrain and influence future actions of entrepreneurs and governmental authorities. Assuming passage through an authorization process as discussed in previous sections (although Canada has not yet – in 2008 – produced its promised – in 2001 – authorization process specific to aquatic GMOs), the introduction of a transgenic salmon would need to compose – beyond the scientific aspects – with environmental, social and economic realities of the sector. Others have suggested ways in which American policy makers could incorporate effective public participation mechanisms within the processes of a regulatory framework (Logar and Pollock, 2005). It has been shown that given appropriate and accessible information, citizens are capable of positive contributions to a reasonable and rational evaluation of advantages and risks. Adjustment of public policy approaches seems to represent the most important modification which would enable attainment of the goal of effective change in this area (Marris et al., 2001). This approach has today to be implemented with the transgenic salmon dossier in order to assess its social necessity and acceptability.

4.5.3. Alternatives

Decision-making should include comparison with alternatives, as in the case of agriculture, it is proposed to evaluate the relative benefits and costs of gene-modified varieties (Schmidt, 2005). For example, the promise of GM rice in China should have been compared with results of a study on thousands of Chinese farmers using agro-ecological techniques. These are based on crop heterogeneity and may represent a solution to the vulnerability of monocultured crops to disease. It has been shown that an agro-ecological approach yielded an increase of 89% productivity while completely eliminating usage of some of the most common pesticides (Zhu et al., 2000). More generally, Chinese farmers using ecologic techniques significantly reduced pesticide use without expensive patented gene-modified seeds (Yanqing, 2002) and this kind of result should also be taken into account when deciding upon use of any GMOs.

Concerns with regard to the safety of aquatic GMOs and the need for careful management of the risks should not prevent the pursuit of research in this area because, firstly, research is needed to determine the nature and severity of risks, and secondly, aquatic GMOs could offer medical or environmental solutions. However, while certain projects may appear to be of interest, the consideration of other parameters may lead to a different overall evaluation. Integration of other factors at the time of the design of new products may make alternatives appear more viable, indeed as better directions of development. Here are two examples to illustrate that, like the European chemical regulation ''REACH'' (EC/1907/2006) or the European Biocides Directive (EC/98/8), for which a process to compare with other solutions (substitution principle or efficiency assessment) should be a part of the assessment:

(1) Let us suppose a salmon to have been modified to enable digestion of more vegetable-based feed, for instance due to one or more enzymes of degradation of vegetable fibres. This could result in change of colour, flavour and odour of the fish as well as its immunizing defences (IFFO, 2001). Biotechnology could study these issues but it remains to be seen whether this would be a financially profitable solution, especially since an alternative solution might be found by simply changing the choice of species, choosing one which is naturally omnivorous or vegetarian.

Another possibility is that biotechnology is used only or also to feed salmons, for instance to colour the fish. Researchers at Fisheries and Oceans Canada (DFO) are exploring the use of molecular technology to develop alternatives to natural or synthetic astaxanthin and canthaxanthin, the classical carotenoid-class antioxidant pigments giving a red–orange colour to the flesh. In addition to modify salmons so that they produce their own pigments, they are investigating the production of GM plants to produce these pigments, but also the improvement of the ability of salmon to take up and deposit these pigments in their flesh, so less expensive quantities would be required in their feeds.

Usually, salmon and shellfish get astaxanthin from eating plankton directly or indirectly, which get astaxanthin from feeding on micro-algae that produce the carotenoid in the first place. Farmed salmon get astaxanthin as a feed additive, both because consumers will not buy white- or grey-fleshed salmon, and because astaxanthin is essential for salmon's growth and overall health (Higuera-Ciapara et al., 2006, vitalchoice.com).

(2) As another example: the promise of a transgenic fish resistant to pathology appears to be of particular interest when some viruses, bacteria or parasites cause very important losses. However, this approach can encounter problems similar to those which are now observed in agriculture. Indeed, experience has shown that transgenic crops, after some years, may engender resistance or new developments in pest species or new parasites, which will occupy the vacant ecological niche. This obliges farmers to increase pesticide treatments to above the quantities used in conventional agriculture (Benbrook, 2004). In fact maintenance of some biodiversity around and in crop

exploitation areas seems a better strategy to fight diseases. This approach could be also preferable in farms where fish density is high.

4.5.4. Labelling

The right of consumers to be informed should also be taken into consideration. Information is essential for consumers to be able to choose between an aquatic genetically modified organism and its non-GM counterpart, and labelling is also a source of security for health and the economy of food and feed industries. This is only possible if information is available when products are sold. For agricultural products in Europe, the Regulation EC/1830/2003 concerning the traceability and labelling of genetically modified organisms, obliges companies to mention any GM ingredient.

4.5.5. Biosafety

Management should integrate biosafety measures during the production process and, post-production, in transport, distribution and consumption. Traceability and monitoring are the tools which would permit operationalization of this objective, and their feasibility has been demonstrated, for GM crops in Europe for instance. Public health and environmental concerns dictate a precautionary approach in this regard, as problems of potentially similar nature, it has been shown, may become manifest only over a lengthy period of time, in geographically distant places, in a succeeding generation or in other persons within a close social network (family, for instance).

A post-monitoring period may be mentioned as well, in that technical advances will surely continue to facilitate detection and, hopefully, diminish costs as well, thus permitting on the one hand detection of risks previously not discernable, and on the other hand to progress scientifically and technically to improve biosafety, notably through containment.

To summarize, given this wide range of issues of concern, governmental intervention and authority appears necessary to guarantee appropriate determination and application of aquatic GMO evaluation and management, with the participation and collaboration of the public and interested parties. Further, it is essential to have international agreement and coordination in place so as to move forward with confidence and trust in areas of international commerce in these new products.

5. Conclusion

The complexity of decisions to be made with regard to aquatic GMOs will result in significant challenges. This will surely contribute to long-term sustainable development with a minimum of external costs. Economic development, environmental protection, and social and health well-being could be advanced through such a shared perspective.

With this objective in mind, we have signaled a broad range of scientific issues and suggested possible guidelines for the evaluation of GM salmon that could inspire all new organism assessment. Complete and in depth research is necessary in order to properly evaluate and validate the innocuity of new,

proposed products considered on a case-by-case basis. This is not performed yet today (ISAAA, 2006). However, it must be remembered that discussion of risk enters into a domain which is not simply reducible to scientific facts. Public consultation must be regarded as an unavoidable way to gain acceptability. This is why democratic decision-making will be furthered by associating as early as possible a broad range of interests, including citizenry, as inclusion of various viewpoints will render the decision-making more robust (Nowotny et al., 2001).

Evaluation of health risks should be done according to tested and recognized scientific methods including a modified long-term oral toxicity test (see Section 4.3), while being based on very complete information of the aquatic GMO. This would include deep knowledge of its genetic construction after transgenesis, whose stability should be assured, but yet still might result in polygenic characters. Evaluation should be characterized by a real will to detect any human and animal health risk, notably by using chronic toxicity tests. The regulation should, furthermore, be able to accommodate a rapidly evolving state of the art in regard to precision in measurements and methods. In any case, progressive and local authorizations for experiments, following international and national guidelines, should be the rule in such cases. Aquatic GMOs may be claimed to reduce environmental risks by incorporating specific genetic features, such as sterility, reduced fitness, inducible rather than constitutive gene expression, and the absence of undesirable selectable markers. Yet the environmental impacts of aquatic GMOs are unverifiable on a theoretical point of view, and they could be permanent and irreversible. The first precautionary principle that dictates not to release unknown engineered aquatic animals into the environment should not be by-passed. Thus environmental risk assessments should necessarily include an artificially confined ecosystem - macrocosm - approach. In addition, it should be kept in mind that the establishment of an aquatic GMO in a new environment depends on its capacities of escape from breeding installations, dissemination, competitiveness for habitat and food, resistance to environmental characteristics and reproductive capabilities. Thus, if authorisations are delivered, monitoring on site and in the area should be systematic.

In summary, decisions about aquatic GMOs should follow some important general principles, starting with transparency with respect to projects, procedures, results of experiments, and decisions. Guarantee of independent expert controls is necessary to assure and maintain citizen confidence in the evolving capacities of public evaluation and control. Other important aspects include implementation of the precautionary principle, adoption of a transdisciplinary, integrated and ecosystemic approach, and evaluation and monitoring of long-term effects of aquatic GMOs. Further, evaluation should concern not only scientific aspects of aquatic GMOs but also alternatives to this new technology, benefits and costs, and broader social aspects, including availability of information for consumers, and issues of concern in North–South and West–East relations. If aquatic GMOs are authorized, environmental monitoring, traceability and labelling for consumers appear to be unavoidable steps towards social acceptability if the citizens are included in the decision process. This is the

price for which industry, citizens and the environment will together benefit from sustainable aquatic biotechnologies.

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