**Outline – Risk assessment of LM fish**

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# BACKGROUND/OBJECTIVE AND SCOPE

\*\*From the AHTEG terms of reference in decision BSVII/12\*\*

*“While revising and improving the Guidance, an attempt should be made to take into account the topics prioritized by the AHTEG, on the basis of the needs indicated by the Parties with a view to moving towards operational objectives 1.3 and 1.4 of the Strategic Plan and its outcomes, for the development of further guidance.”*

\*\*From the report of the AHTEG meeting 2015\*\*

*The AHTEG decided to recommend to the COP-MOP the development of additional guidance on ‘risk assessment of LM fish’ and ‘risk assessment of LMOs produced through synthetic biology’. The Group will prepare outlines on the two topics for the COP-MOP in order to facilitate its consideration and further development of the topics as separate guidance.*

We were asked to draft an outline for standalone guidance on risk assessment of LM fish in accordance with the Cartagena Protocol. This outline will be submitted to the COP-MOP at its meeting in December 2016, and the Parties will decide whether or not this guidance is needed and, if so, how it could be developed.

The current draft for a risk assessment document of LM fish includes all freshwater, marine and anadromous fish and shellfish, including aquarium species. It its current form, this draft guidance focuses on aspects that are unique or particularly relevant to LM fish and is meant to be complemented by the Roadmap.

# INTRODUCTION

LM fish are produced for a variety of purposes, including growth-enhancement for human food production in aquaculture, biological control of nuisance species, recreational fishing, monitoring water quality to detect contaminants, as bio-factories to produce commercially valuable compounds such as human pharmaceuticals, and ornamental aquarium market.

## Overview of LM fish under development

See table in annex1

## Issues that are unique or particularly relevant to RA of LM fish

* Highly mobile and live in aquatic environments
* Potential to escape from containment facilities and spread to natural environments
* Non-LM counterpart may be protected by national law, for example several countries protect species of wild salmon
* Phenotypic plasticity
* Genetic variability

## Uncertainties

* Limited understanding of the whole genome of fish species, for example due to lack of genomic, proteomic and metabolomic information
* Lack of historical data regarding the environmental fate of transgenes and novel genetic elements in LM fish
* Lack of empirical evidence regarding invasiveness of LM fish, for example LM fish with enhanced fitness
* Will the migratory and mating behavior of LM fish remain the same as compared to their non-modified counterparts?
* Will the habitat range of LM fish with improved tolerance to biotic or abiotic stresses remain the same as their non-modified counterparts? Will the ~~reversibility~~ irreversibility of sterility/ infertility be fully effective?

## Case-by-case

As for other LMOs, the case-by case approach must also be applied to the risk assessment of LM fish. This guidance will not focus on one particular method of generation of LM fish, receiving environment, intended use or species. Therefore, the risk assessment criteria and requirements will not be equally relevant in all cases. Below are some elements to consider:

* ~~The characteristics of the non-modified parental organism~~
* Modified genetics elements or inserts ~~The inserted transgenes~~
* The altered traits of the LM fish (including target and non-target traits)
* The accessible environments, i.e. environments that the LM fish may enter accidentally or into which they may be deliberately introduced
* The intended uses (including scale and temporary nature of the (confined) release).

# PLANNING PHASE OF THE RISK ASSESSMENT

## The choice of comparators

* Parental line and wild fish
* Experience and history of use taking into account its ecological function.
* Centers of origin and centers of genetic diversity.
* Current distribution areas & habitats where wt type organisms may persist or proliferate.

## Problem formulation

Protection goals, assessment endpoints

Theories on predicting the environmental fate of transgenes or the transgenic individual: purging, spread, Trojan gene, disappearance, establishment to answer the questions: is it ecologically safe, alter genetic diversity, harm species of special concern or reduce aquatic biotic community resilience?

# CONDUCTING THE RISK ASSESSMENT

## Vertical and horizontal gene transfer (see roadmap step 1, step 2 and 3) in the potential receiving environment.

* Survival of DNA from LM fish in water (feces and from decaying dead fish)
* Survival of DNA from LM fish bound to particles in water and to sediments
* Spread of transgenes to wild or feral relatives
* Spread of transgenes to wild or feral relatives of a native species
* Spread of transgenes to wild or feral relatives of an alien species already established in the ecosystem
* Spread of antibiotic resistance genes ~~used in the genetic modification process~~
* Heightened invasiveness by an alien species due to one or more traits altered by the modification.
* Ecological, evolutionary and stochastic factors that could affect the fate of transgenes
* Harm to the gene pools in the affected species’ centre of origin and/or diversity

## Risk evaluation of potential hybrids (see roadmap step 1)

## Crosses of LM fish made by different biotechnological techniques (see roadmap step 1)

For example in the future it may be triploid fish that is crossed with LM fish. The fact that a fish is triploid may also add on uncertainties with regard to environmental impact (triploid fish grows faster and are larger than diploid fish).

We find it difficult to set a limit on this point. Regarding the uncertainties, we consider that the offpring of LM fish with other fertile fishes made by the use of other biotechnological techniques should be assessed in the context of the LM fish. If we consider other differences separately, we could also take into account differences that could arise as a consequence of crossing LM fish with one obtain traditional breeding (e.g, the genetic background could alter the phenotype). At least in our country there is no regulation on non-LM triploid fish. We suggest not considering this point.

## Testing the living modified fish in representative environments (see roadmap step 1)

Regional variation and differences in the environment may influence the characteristics and the behavior of LM fish. Experimental trials should be performed in as representative condition as possible.

## The likely potential receiving environment(s) (see roadmap step 1, step 2 and step 3)

The identification and characterization of likely potential receiving environments may be dependent on several factors, and the potential for dispersal into potential environment is important to consider.

## Persistence and invasiveness (see roadmap step 1, step 2 and step 4)

* Will metabolism and/or other biological parameters remain unchanged for LM fish? If they are different, how will they affect growth, fish health/welfare?
* The net ﬁtness trait data on real transgenic individuals and their non-engineered counterparts. Six ﬁtness components (fecundity, fertility, juvenile viability, age at sexual maturity, mating success, and longevity)

## Dispersal mechanisms (see roadmap step 1 and step 2)

LM fish have a variety of ways to reproduce and this is relevant for a risk assessment.

## Target/non-target organisms (see roadmap step 2, 3 and 4)

Harm to species of special concern, such as endangered species or economically or culturally important species

## Fish pathogens, infections and diseases (see roadmap step 3)

May LM fish that are resistant to fish pathogens, infections and diseases can be carriers of the same diseases and hence by escpace spread the same diseases?

In general an escape by such fish will most probably follow the Spread hypothesis.

## Unintentional transboundary movements (article 17)

Fish have a broad geographical distribution, although that will vary depending on the species. Confinement will be dependent on the species and the strategy to develop LM fish.

## Risk management strategies (see roadmap step 5)

What can be done (including bio-conﬁnement and other conﬁnement) to reduce risk, either by reducing the likelihood of and implementation the harm occurring or mitigating the potential effects in the event that it does occur?

Monitoring methods

* Baseline information
* Traceability mechanisms of LM fish
* How effective are the implemented measures for risk reduction?
* What follow-up, corrective action or intervention will be pursued if ﬁndings are unacceptable?
* Did the intervention adequately resolve the concern?

## Containment strategies of LM fish (see roadmap step 2 and 5)

*Physical containment:* first line of defense in preventing the escape of transgenic fish, for example net pens and sea cages. Geographically isolating an aquaculture facility, such as in a closed recirculating landlocked site or a natural body of water that it is not close to waterways or other bodies of water.

*Physicochemical containment:* physicochemical measures are designed to induce 100% mortality in one or more specified life stages through lethal condition changes in the water environment. These include temperature change, changes in pH, or the treatment of effluent water with dissolved chemicals, such as chlorine, bromine, or ozone, to kill any potential transgenic escapees.

*Reproductive containment:* the best safeguard against the spread of transgenes would be to render transgenic fish completely sterile. Thus far, the most developed, effective, and widely documented scientific method for the reproductive confinement of transgenic fish involves disrupting sexual reproduction by triploidy induction. Physical induction of triploidy involves the precise application of hydrostatic pressure, temperature, or electrical shock or chemical treatment at a specific time after egg fertilization or crossing of tetraploid fish with diploid fish.

*Method of induction of sterility in fish ~~and their efficacy~~:* Transgenic sterilization; inactivation targets to induce sterility and transgenic inactivation approaches for sterility. Transgenic disruption of embryonic development. Gonad-specific transgenic excision. DNA vaccination to disrupt sexual maturation. The use of CRISP/Cas to knock out essential proteins necessary for gamete production.

# RELATED ISSUES

* New emerging technologies used to make LM fish
* The combination of different techniques for making LM fish, like triploidy induction together with genetic modification techniques
* Ecological resilience of aquatic biological communities – their ability to recover from external disturbances such as ﬂoods, contaminants or climate change
* Focusing on LM fish intended for biopharming, potential adverse effects of the incidental exposure of humans or other animals to the LM fish, such as toxic or allergenic effects.

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# ANNEX

**Annex 1. Examples of genetically engineered ﬁsh and shellﬁsh under development:**

|  |  |  |  |
| --- | --- | --- | --- |
| Purpose | Species | | Target Engineered trait |
| Aquaculture (human food) | Finﬁsh | Atlantic salmon | Increased growth rate and food conversion efﬁciency by inserting Chinook salmon growth hormone gene and antifreeze gene promoter |
| Channel catﬁsh | Enhanced bacterial resistance after insertion of moth peptide antibiotic, cecropin B gene |
| Grass carp | Increased resistance to grass carp haemorrhage virus after insertion of human lactoferrin gene |
| Goldﬁsh | Increased cold tolerance after insertion of ocean pout antifreeze protein gene |
| Molluscs | Oysters | Improved disease resistance by inserting retroviral vectors with disease resistance genes |
| Crustaceans | Crayﬁsh | Various aquaculture production traits by injection of replication-defective pantropic retroviral vector. Success in producing transgenic individuals shown by expression of marker gene |
| Hobby aquarium market | Finﬁsh | Zebraﬁsh | Fluorescent red or green body colour |
| Pharmaceutical production | Finﬁsh | Tilapia | Production of clotting factor after insertion of human gene for clotting factor VII, for medicinal applications |
| Biological control of aquatic nuisance species, such as common carp | Finﬁsh | Carp andmedaka | Production of male-only offspring by insertion of gene construct that prevents the ﬁsh’s aromatase enzyme from transforming reproductive hormone androgen into oestrogen; to prevent development of female ﬁsh |
| Industrial andenvironmental uses | Finﬁsh | Medaka | Transgenic ﬁsh serve as a detector of mutations (presumably caused by pollutants) that could affect aquatic animal or human health. After insertion of mutagenic bacteriophage vector, vector deoxyribonucleic acid (DNA) is removed and inserted into indicator bacteria to measure mutant genes |