**Outline – Risk assessment of LM fish**

BACKGROUND/OBJECTIVE AND SCOPE 2

INTRODUCTION 3

Overview of LM fish under development 3

Issues unique for RA of LM fish 3

Uncertainties 4

Case-by-case 4

PLANNING PHASE OF THE RISK ASSESSMENT 4

The choice of comparators 4

Problem formulation 4

CONDUCTING THE RISK ASSESSMENT 5

Vertical and horizontal gene transfer (see roadmap step 1, step 2 and 3) 5

Risk evaluation of potential hybrids (see roadmap step 1) 5

Crosses of LM fish made by different biotechnological techniques (see roadmap step 1) 5

Testing the living modified fish in representative environments (see roadmap step 1) 5

The likely potential receiving environment(s) (see roadmap step 1, step 2 and step 3) 5

Persistence and invasiveness (see roadmap step 1, step 2 and step 4) 6

Dispersal mechanisms (see roadmap step 1 and step 2) 6

Target/non-target organisms (see roadmap step 2, 3 and 4) 6

Fish pathogens, infections and diseases (see roadmap step 3) 6

Unintentional transboundary movements (article 17) 6

Risk management strategies (see roadmap step 5) 7

Containment strategies of LM fish 7

RELATED ISSUES 8

BIBLIOGRAPHIC REFERENCES 8

ANNEX 8

# 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.

# My suggestion is to added paragraph:

**Objective and scope.** In this paragraph can be specified that “this guidance will include all freshwater, …….” .

My further suggestion is that the document shall include not only anadromous fish but also catadromous, thus could be specified that it will include diadromous fishes.

Furthermore shall be specified if the guidance refers to contained use, such as aquaculture, “stocking” (defined as “the deliberate release of fish into the wild at any stage of their life-cycle for enhancement, mitigation, restoration, rehabilitation or ranching purposes), or any other use.

## Overview of LM fish under development

See table in annex1.

* For a common and better comprehension the scientific name shall be reported in the table: i.e. Atlantic salmon (Salmo salar; Salmonidae) and common carp (Cyprinus carpio; Cyprinidae); medaka (Oryzias latipes); zebrafish (Danio rerio); tilapia

(Oreochromis spp.), etc..

* Indication on the state of art (i.e.research level or field trials) could be added to better evaluate the potential contents of further guidance or, at least, web links to databases could be suggested.

## 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 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, 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
* 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

# PLANNING PHASE OF THE RISK ASSESSMENT

## The choice of comparators

* Parental line and wild fish: For non-indigenous species, provide the available information on the proposed species’ life-history, preferred habitat, potential parasites and disease agents, and potential for competition with Atlantic salmon in the recipient waters or nearby waters.
* Details of the available biological characteristics of comparator population. This would include such characteristics as run timing, time of spawning, age-at- maturity, size-at-age, etc. and potential for competition with local populations of the LM fish in the recipient waters or nearby waters.

## Problem formulation

Protection goals, assessment endpoints

~~Theories~~ Hypothesis 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 and gene pool of the interested specie, harm species of special concern or reduce aquatic biotic community resilience?

Problem formulation shall take into account the final use of the LM fish, i.e. research, stocking, farming, biocontrol, replacement of native food resources. This would help during the evaluation of consequences and exposure (something similar to the box of the road map “Information requirements in the case of field trials or experimental release” line 299 of the road map).

# CONDUCTING THE RISK ASSESSMENT

As in the Risk Assessment of Living Modified Mosquitoes of the Road map, a paragraph on “Characterization of the living modified fish” could be added, together with paragraph “Unintended effects on biological diversity (species, habitat, ecosystem, and ecosystem function and services)”. In this last paragraph, the information reported in the points of the draft\_outline: “Crosses of LM fish made by different biotechnological techniques”, “Persistence and invasiveness”, “Target/non-target organisms” and “Fish pathogens, infections and diseases” can be included. Moreover the paragraph “Elements for consideration” could be added.

## Vertical and horizontal gene transfer (see roadmap step 1, step 2 and 3)

* 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 relatives of a native species
* Spread of transgenes to 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

## 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).

## 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.

Shall be specified that experimental trials should be done in confined sites.

In my opinion this paragraph shall follow the paragraph “The likely potential receiving environment(s)”.

## 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.

For each species of LM fish the River Classification and Zoning process could be applied ( appendix 1 of Williamsburg Resolution)

Specific zone of release should be identified and characterized, taking into account the LM species or purpose of the release,.

Information on species composition at proposed site of introduction and adjacent rivers shall be provided, together with information on other releases and/or uses in the environment and/or region for the trials or activities.

This requests are already foreseen in the Williamsburg Resolution. This document is related to the Conservation of Salmon in the North Atlantic Ocean, but could be adapted to other fish *genera* and *familia*e (<http://www.nasco.int/pdf/agreements/williamsburg.pdf>.)

## 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) Annex 3

## Some suggestions can be taken from Guidelines on Containment of Farm Salmon, CNL(01)53- of the Williamsburg Resolution.

## Furthermore RM Strategies normally used in aquaculture and/or fish farming can be applied, such as:

## distance from rivers or waters where the conventional counterpart lives or from sites of reproduction and or gene pool natural bank. “The separation distance between aquaculture facilities at marine sites should be based on a general assessment of local conditions. Wherever possible, different generations of salmon should be reared in separate locations.” (<http://www.nasco.int/pdf/agreements/williamsburg.pdf>

## Parties should consider the establishment of gene banks for stocks that are in danger of extirpation. This could provide a source of genetic material for future restoration programs.

## A site-specific contingency plan shall be developed for use when an escape event occurs.

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

* 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

*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

# BIBLIOGRAPHIC REFERENCES

Cowx I.G., Bolland J.D., Nunn D., Kerins G., Stein J., Blackburn J., Hart A., Henry C., Britton J.R., Copp G., Peeler E. (2010) Defining environmental risk assessment criteria for genetically modified fishes to be placed on the EU market. Scientific/technical report submitted to EFSA, CT/EFSA/GMO/2009/01, 1-264 -<http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/69e.pdf>

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2013. Guidance on the environmental risk assessment of genetically modified animals. EFSA Journal 2013;11(5):3200, 190 pp. doi:10.2903/j.efsa.2013.3200. <http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3200.pdf>

Kapuscinski et al. 2007 Environmental Risk Assessment of Genetically Modified Organisms Volume 3: Methodologies for Transgenic Fish, published by CAB International. <https://bch.cbd.int/database/record.shtml?documentid=44302>

**Suggested bibliographic references**

CNL(06)48:Resolution by the Parties to the Convention for the Conservation of Salmon in the North Atlantic Ocean to Minimise Impacts from Aquaculture, Introductions and Transfers, and Transgenics on the Wild Salmon Stocks -the Williamsburg Resolution(Adopted at the Twentieth Annual Meeting of NASCO in June 2003 and amended at the Twenty-First Annual Meeting of NASCO in June 2004 and at the Twenty-Third Annual Meeting of NASCO in June 2006).<http://www.nasco.int/pdf/agreements/williamsburg.pdf>

John A. Beardmore and Joanne S. Porter. 2003 Genetically Modified Organisms and Aquaculture. FAO Fisheries Circular No. 989 FIRI/C989(En). <http://www.fao.org/docrep/006/y4955e/y4955e00.HTM#Contents>

# 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 and environmental 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 |