# Attempt to link paternal genotoxic exposure to reproductive impairment in fish

Luc Fiat, Christian C. Gillet, Sylvie Bony, Emilie Lacaze, Alain Devaux

14th INTERNATIONAL SYMPOSIUM ON TOXICITY ASSESSMENT, August 30 to September 4, 2009, Metz, France

# ATTEMPT TO LINK PATERNAL GENOTOXIC EXPOSURE TO REPRODUCTIVE IMPAIRMENT IN FISH

Luc Fiat[1], Christian Gillet[2], Sylvie Bony[1], Emilie Lacaze[1] and Alain Devaux[1]

email : alain.devaux@entpe.fr

[1] Université de Lyon, INRA-LSE, ENTPE, rue Maurice Audin, F-69518 Vaulx-en-Velin Cedex, France
[2] INRA, UMR CARRTEL, avenue de Corzent, F-74203 Thonon les Bains, France

## *INTRODUCTION*

The aquatic environment is becoming increasingly contaminated by pollutants having a genotoxic potential towards organisms and fish in particular. Such genotoxins are prone to affect directly fish larvae or indirectly reproductive process. This could influence recruitment rate and hence the population dynamics (Newman and Clements, 2008). However, assessment of the ecological risks associated with environmental genotoxic exposure is generally individual based. Thus, there is a need for a better understanding of the long term and population level implications of such genotoxic exposures, by studying genotoxic impact on fish reproduction. The present work aims to track the transfer of exposure effects across fish generations by studying the link between the level of DNA damage in spermatozoa of male fish exposed to the model genotoxicant MMS and the rate of abnormalities measured in the offspring.

## *EXPERIMENTAL DESIGN*

• **Organisms and exposure**:

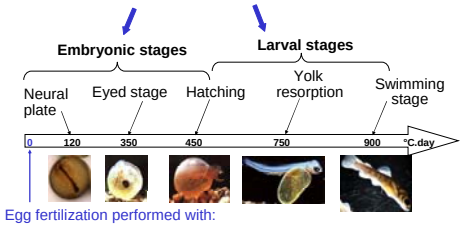
Brown trout Arctic charr

**Mature male** brown trout and Arctic charr were intraperitoneally injected with MMS (50mg/kg body weight in coconut oil) and sperm was collected by stripping after 3 weeks of exposure.

• **Fertilization and Comet assay procedure**:

Pool of eggs collected from **unexposed female** trout and charr were fertilized with control or exposed fish sperm and then incubated to follow their development.
Alkaline version of the Comet assay was performed on spermatozoa according to the procedure described by Singh *et al*. (1988).

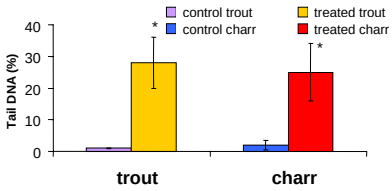
• **Development assessment at different stages**:




Egg fertilization performed with:
- 18 control and 28 MMS-exposed charr sperm
- 16 control and 31 MMS-exposed trout sperm

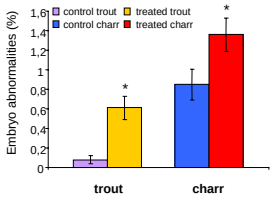
## *MAIN RESULTS*

• **Sperm DNA damage**




Sperm DNA of both trout and charr was significantly damaged after the 3 week MMS treatment

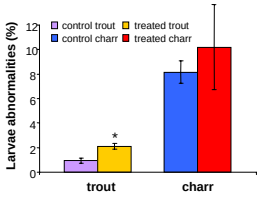
• **Fertilization rate and embryo development**




- Fertilization rate was not affected by MMS treatment
- Embryo abnormalities clearly increased after male parent exposure in both species

• **Larvae development**


Male exposure to MMS led to a large array of abnormalities in larvae
A and E: yolk oedema in trout and charr respectively; B and C: trout spine deformation;
D: charr jaw deformation; F: charr Siamese larvae



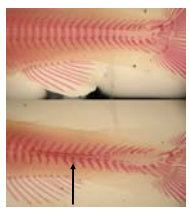

Larvae abnormality rate was significantly higher in trout after male parent exposure

• **Skeleton development**

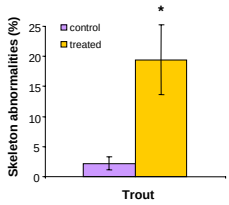
Since trout larvae exhibited significantly enhanced abnormalies after male parent exposure, larvae were fixed using formaldehyde and bony structures were examined under microscope after alizarin red S staining.

control

MMS treated



Spine deformation




Skeleton abnormalities (mainly spine and cephalic deformations) reached a very high level in larvae stemming from MMS treated male parent

## *DISCUSSION & PERSPECTIVES*

Although no statistical significant correlation between the level of sperm DNA damage and the occurrence of offspring abnormalities was demonstrated, exposure of male trout and charr for 3 weeks to the model genotoxicant MMS clearly led to an increase in both these parameters. First, the present work shows the interest of studying the impact of genotoxic compounds on **spermatozoa,** a cell type that exhibits a high genotoxic response, possibly due to low DNA repair and low biotransformation capacities contrary to oocytes. Consequently, spermatozoa are susceptible to accumulate DNA damage under chronic and low-dose exposure to environmental genotoxins. Second, present results highlight the possible **transfer of genetic damage from adult to offspring** of freshwater fish as recently demonstrated in marine and freshwater invertebrates (Lewis and Galloway, 2009; Lacaze *et al*., 2009).
The rate of abnormalities increases along the offspring development time: this stresses the interest to further study integrated responses such as fish growth, survival and F2 reproductive success. Moreover, it would be necessary to take into account both the oocyte capacity to repair spermatozoa DNA damage, and, the contribution of a potential oocyte DNA damage to the observed reproduction impairment.
This work represents a first step in understanding the functional significance of genotoxic damage in fish germ cells, revealed as a **reproductive failure**.

**Acknowledgments:**
- Philippe Laurent, INRA , UMR CARRTEL Thonon les Bains
- François Meunier, Laboratoire d'Ichtyologie, Muséum national d'histoire naturelle de Paris

**References:**
-Newman M.C. and Clements W.H., Ecotoxicology: a comprehensive treatment, CRC Press (2008), 252 pp.
-Singh *et al.*, Exp. Cell Res. 175 (1988) 184-191
-Lewis C. and Galloway T., Environ. Sci. Technol. 43 (2009) 928-933
-Lacaze *et al.* (2009), oral presentation during ISTA14