Ecotoxicological significance of genotoxicity in aquatic species germ cells

Alain Devaux[1], Sylvie Bony[1], Christian Gillet[2], Emilie Lacaze[1], Luc Fiat[1], Raphaël Santos[1,3], Wilfried Sanchez[3], Jean-Michel Olivier[1]

[1] USC INRA IGH, UMR 5023 LEHNA, ENTPE, Vaulx en Velin
[2] INRA UMR CARRTEL, Thonon les Bains
[3] INERIS, Unité d'Ecotoxicologie, Verneuil en Halatte

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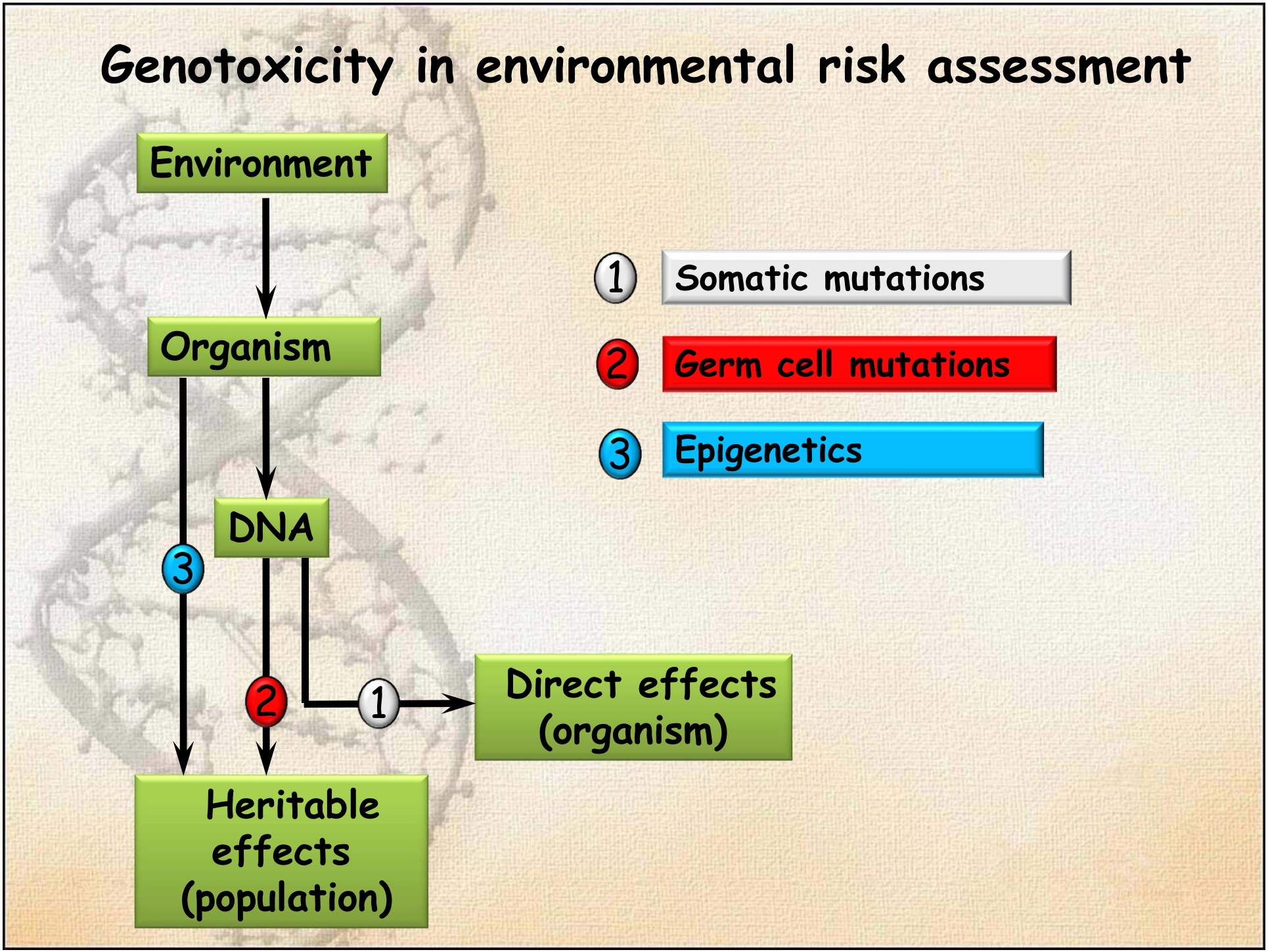
Aquatic ecosystems:

- ultimate receptacle for anthropogenic compounds
- 1/3 could have genotoxic properties
- great progress in understanding the implications of genotoxin exposure to human health

- BUT there is a lack of knowledge regarding the significance of genotoxicity impacts on aquatic species…

Genotoxicity in environmental risk assessment

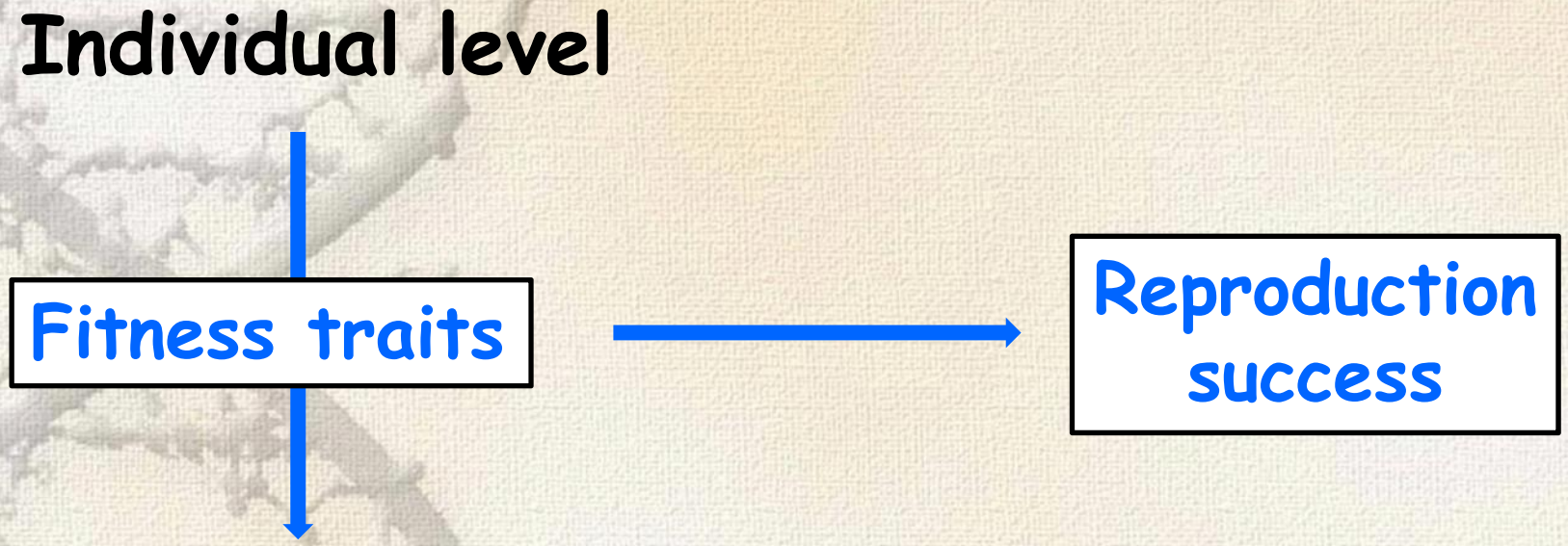


Genetic ecotoxicology:

- gamete loss (cell death)
- embryo mortality (lethal mutations)
- abnormal development
- neoplasia
- heritable mutations affecting genetic diversity and gene expression

and finally Darwinian fitness

A relevant goal in ecotoxicology → **To relate effects manifested in individuals to changes in population size or structure**



Anderson S.L & Wild G.C, 1994. Linking genotoxic responses and reproductive success in ecotoxicology. Environ. Health Perspect. 102: 9-12

Depledge M.H., 1998. The ecotoxicological significance of genotoxicity in marine invertebrates. Mutat. Res. 399: 109-122

DA1

Genotoxic damages in germ cells can be passed on to future generations if not or bad repaired :

Do they influence the recruitment rate and therefore the population dynamics ?

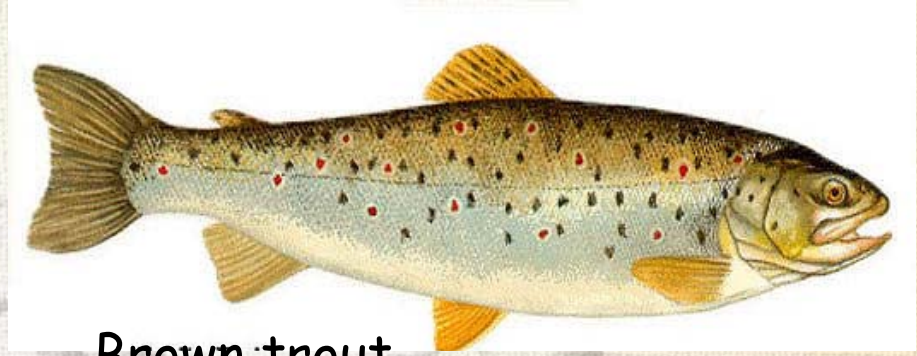
Our proposal:

Try to fill this gap by studying genotoxic impact on aquatic species reproduction after parental exposure

Diapositive 6

DA1 I will illustrate it by two case-studies carried out on two aquatic species along the food web (fish and crustacean)

DEVAUX Alain; 20/08/2009



Brown trout
(*Salmo trutta*)

-Northern and middle Europe
-rivers, lakes, sea shores
-reproduces in cold and
low polluted water

Arctic charr
(*Salvelinus alpinus*)

-Alpine area
-oligotrophic lakes
-reproduces in cold, deep
and low polluted water

Experimental design

trout and charr males
exposed for 3 weeks to MMS (50mg/kg)

trout and charr females
unexposed

Sperm

1 pool of eggs

Spermatozoa
DNA damage
(Comet assay)

Fertilization

Embryonic stages

Larval stages

Neural plate

Eyed stage

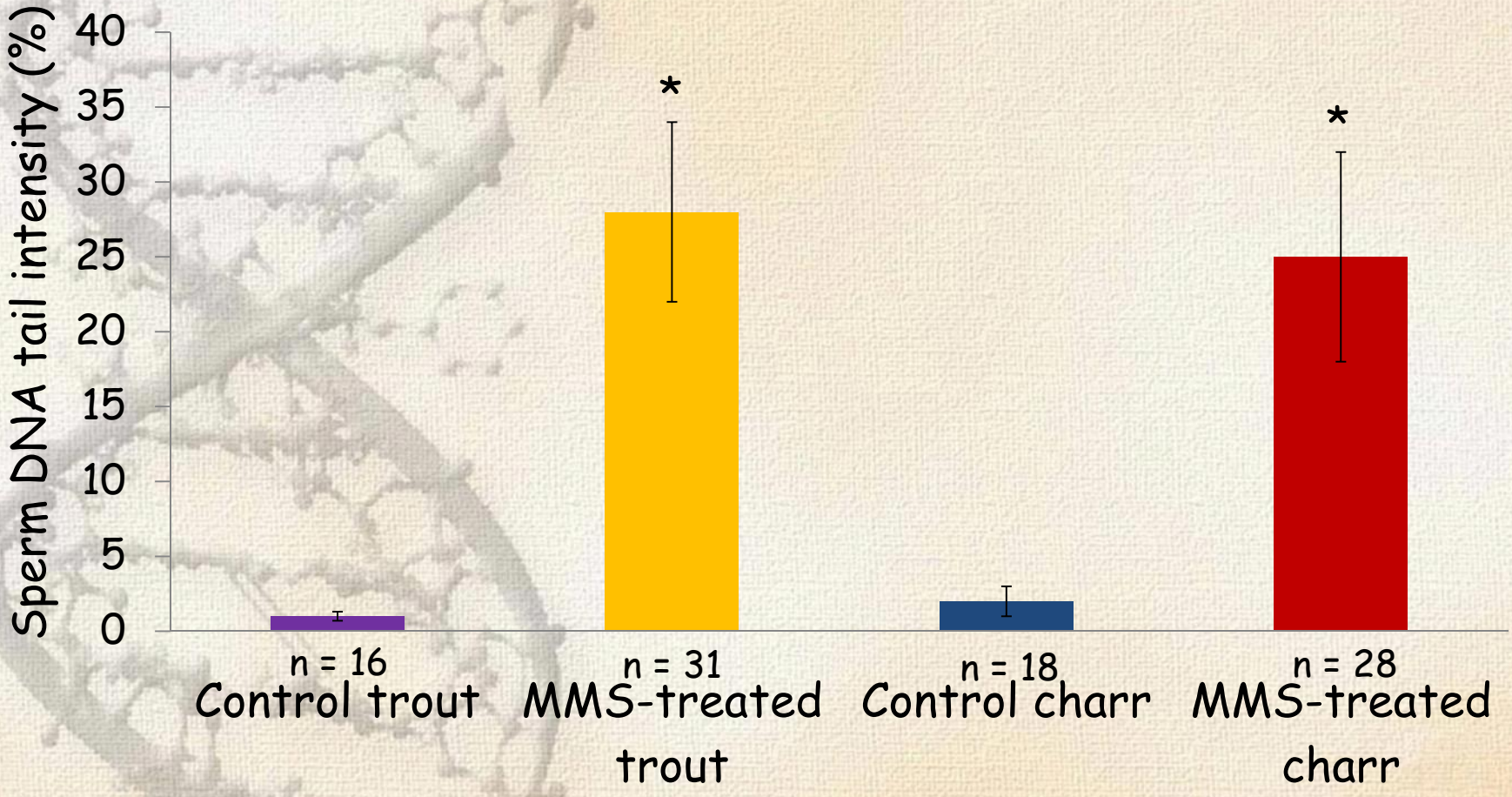
Hatching

Yolk resorption

Swimming stage

Fertilization → **120 days**

Sperm DNA damage (mature spermatozoa)



	Fertilization rate (%)	
	Trout	Arctic charr
Control males	94 ± 5	84 ± 6
MMS-treated males	95 ± 5	83 ± 6

Sperm DNA of fish significantly damaged after 3 week MMS treatment, without any effect on fertilization rate

Embryo development impairment

Neural plate

Eyed stage

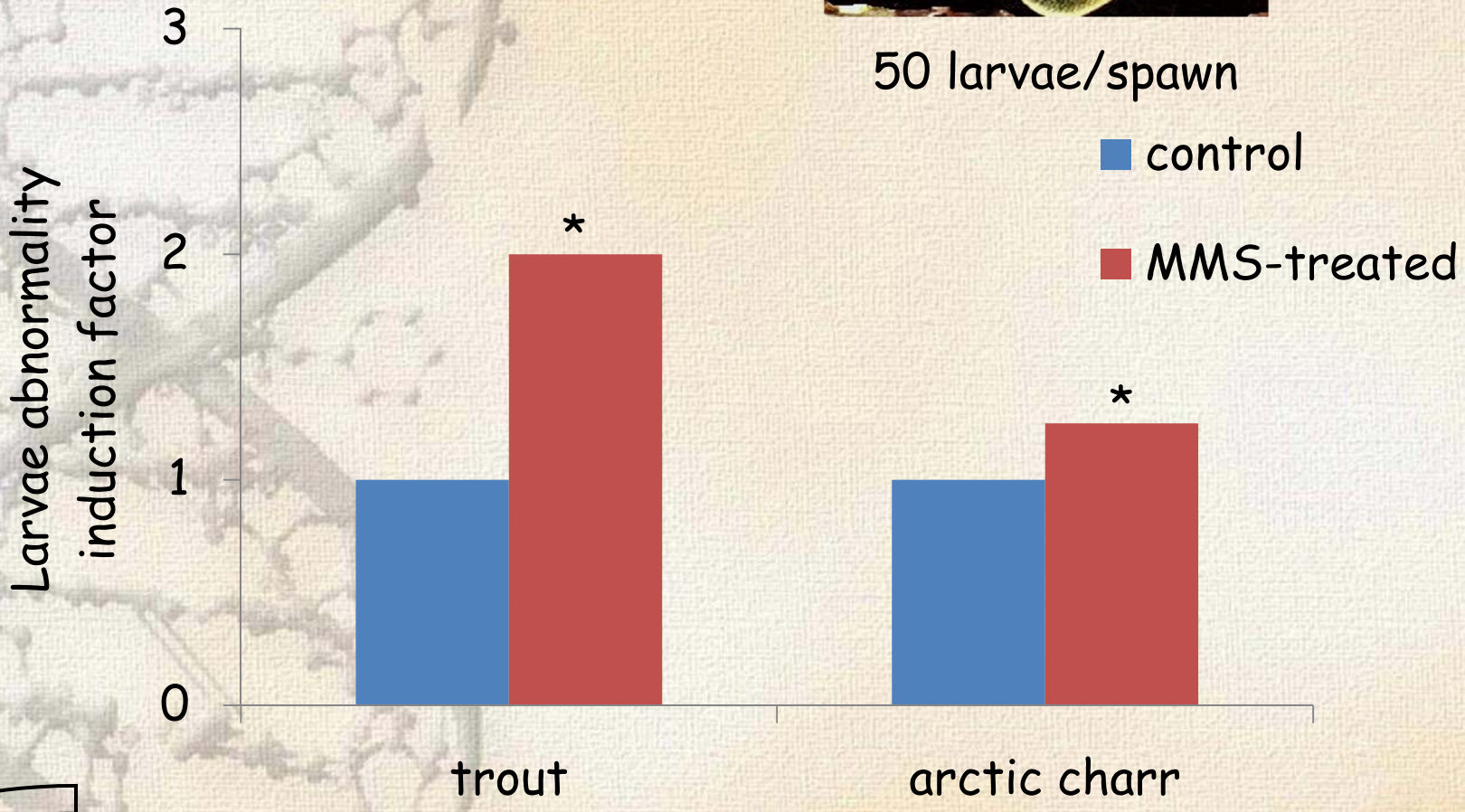
Hatching

Embryo abnormality rate increased in fry from both species

Larvae development impairment

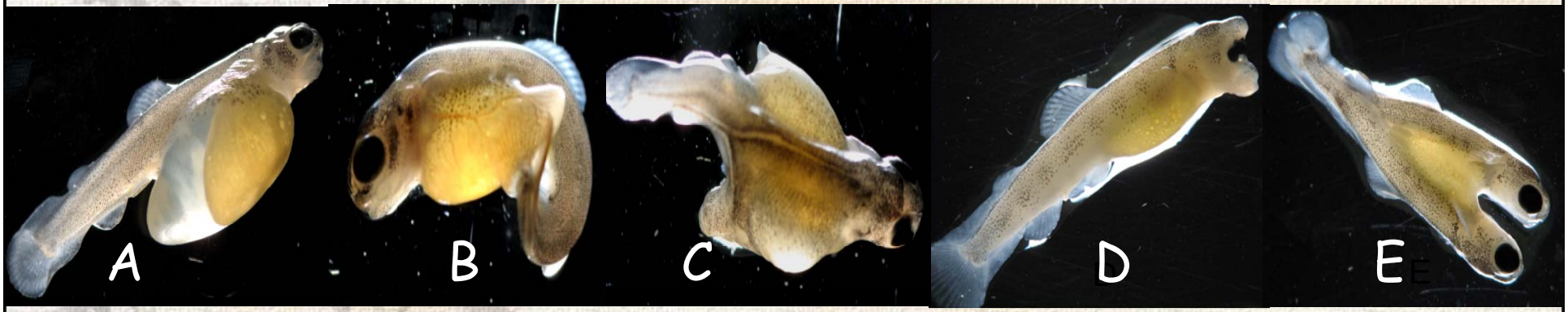
Yolk resorption

50 larvae/spawn



Male exposure to MMS led to a large array of abnormalities, mainly in trout larvae

Some examples of larvae abnormalities



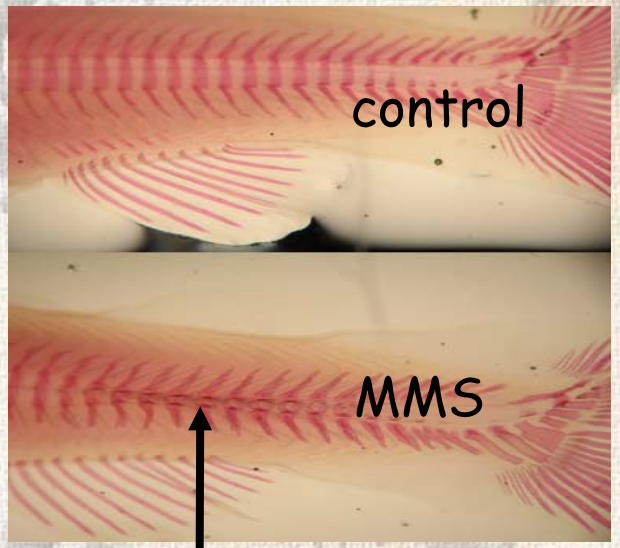
A: yolk oedema

B and C: spine deformation

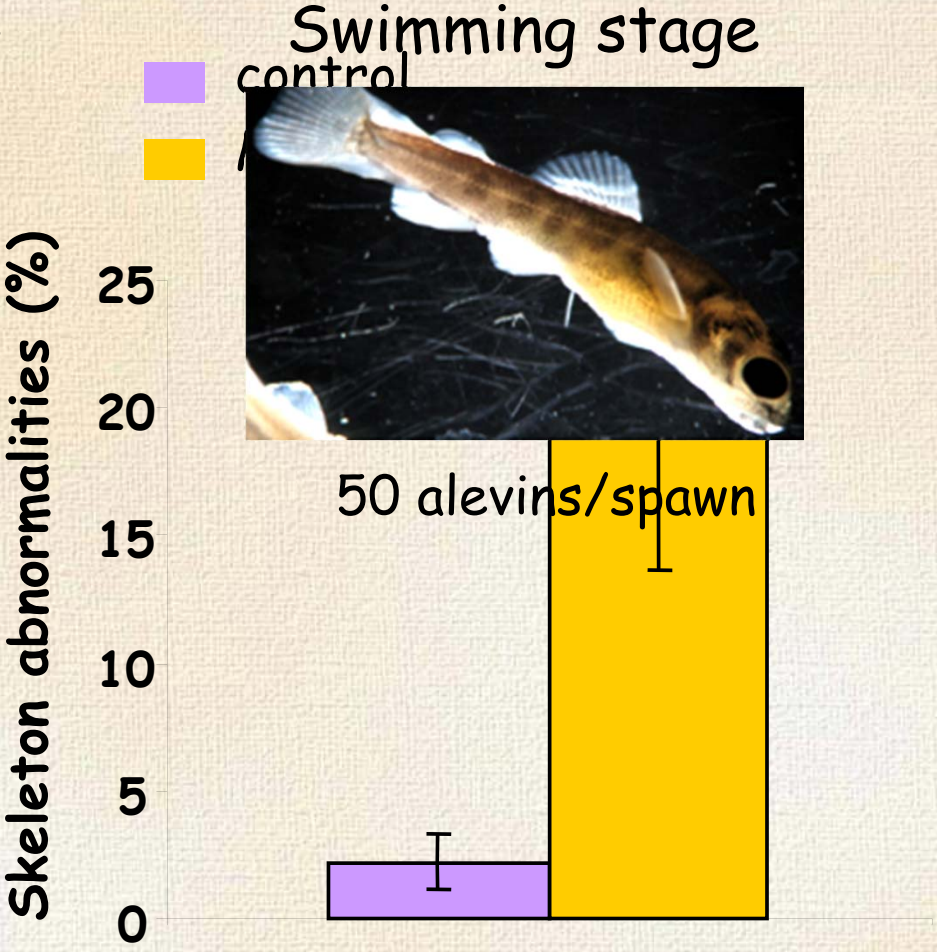
D: jaw deformation

E: Siamese larvae

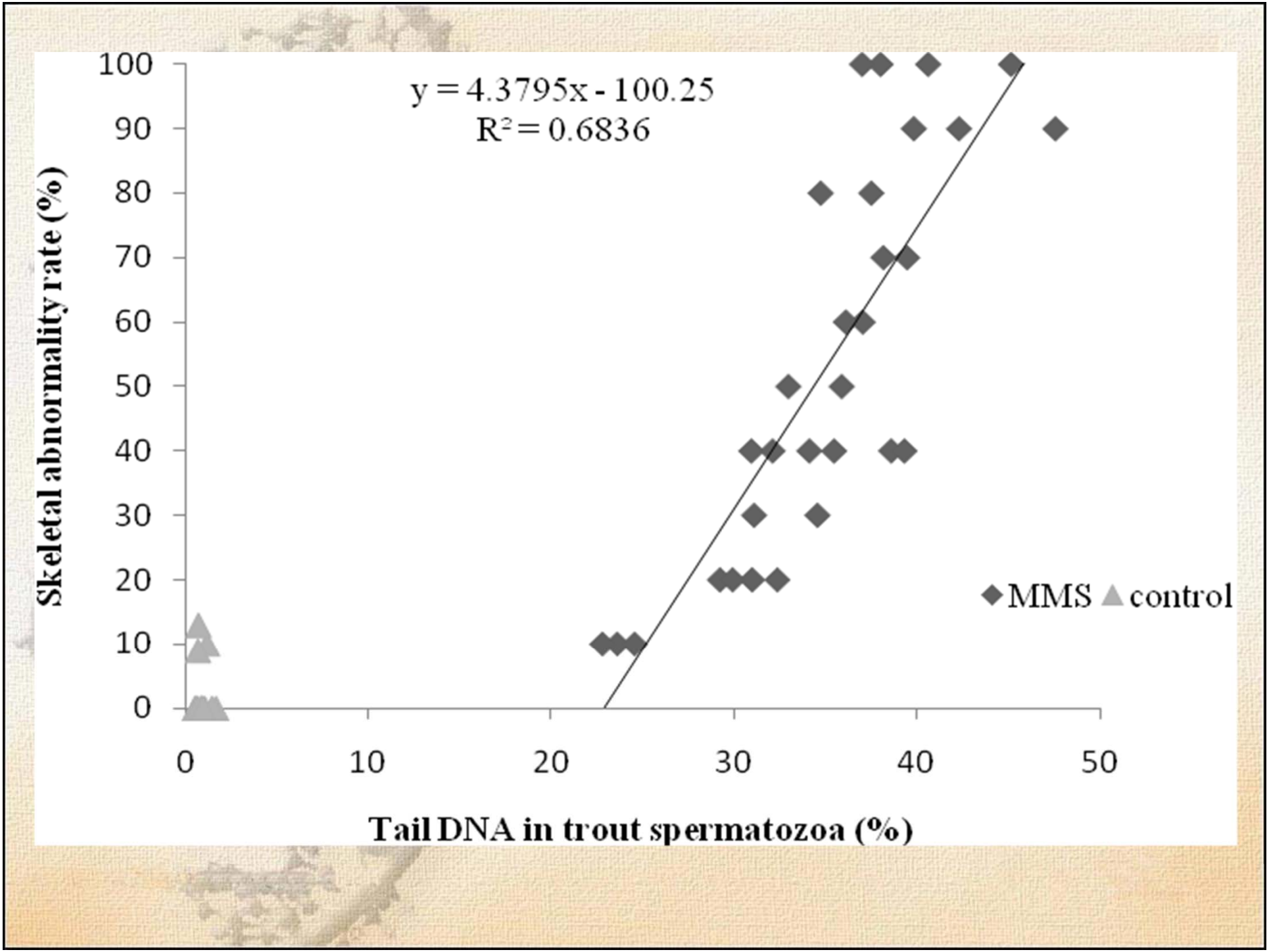
Skeleton development impairment in trout



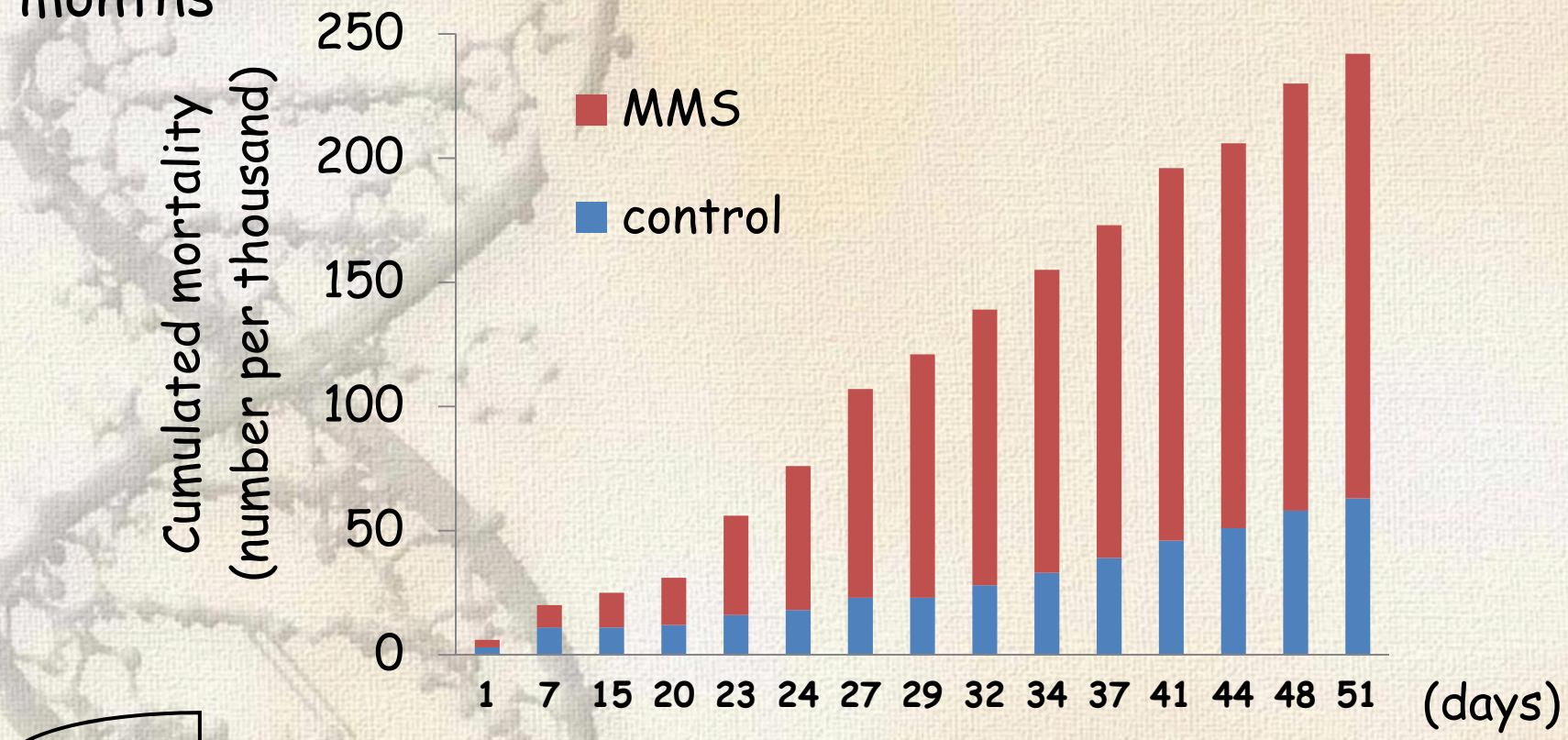
Spine deformation



Skeleton abnormalities (mainly spine and cephalic deformations) reached a very high level in trout larvae stemming from MMS treated male (10-fold increase, around 20 %)

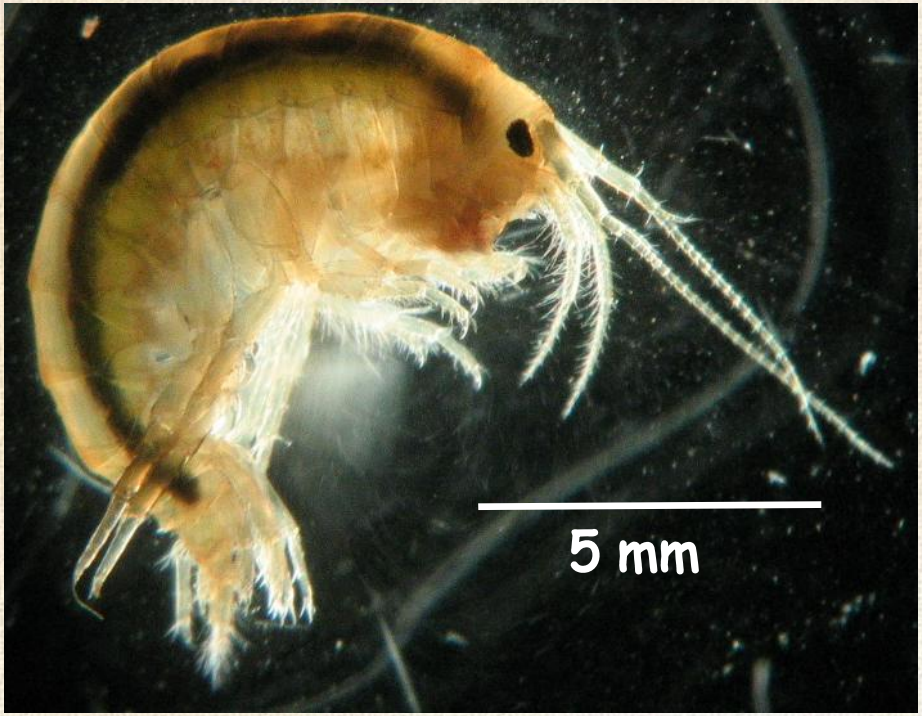

y = 4.3795x - 100.25
R² = 0.6836
Skeletal abnormality rate (%)
Tail DNA in trout spermatozoa (%)
MMS
control
0
10
20
30
40
50
60
70
80
90
100

After the swimming stage, trout alevins from different spawns are gathered and survival is monitored for 2 months

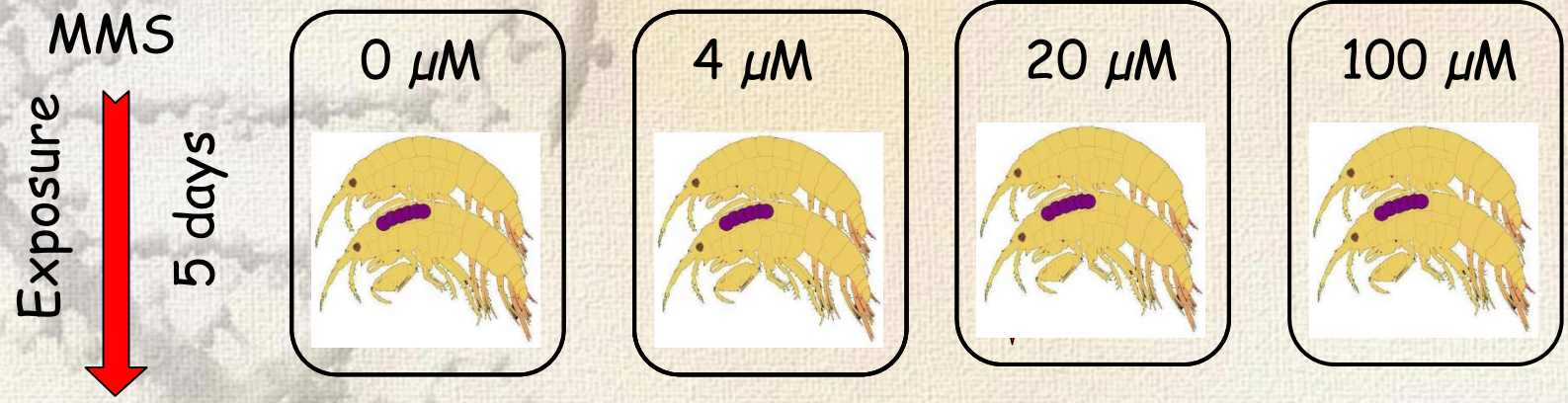


After 2 months, mortality in trout offspring from treated male reached a 5-fold value compared to the control

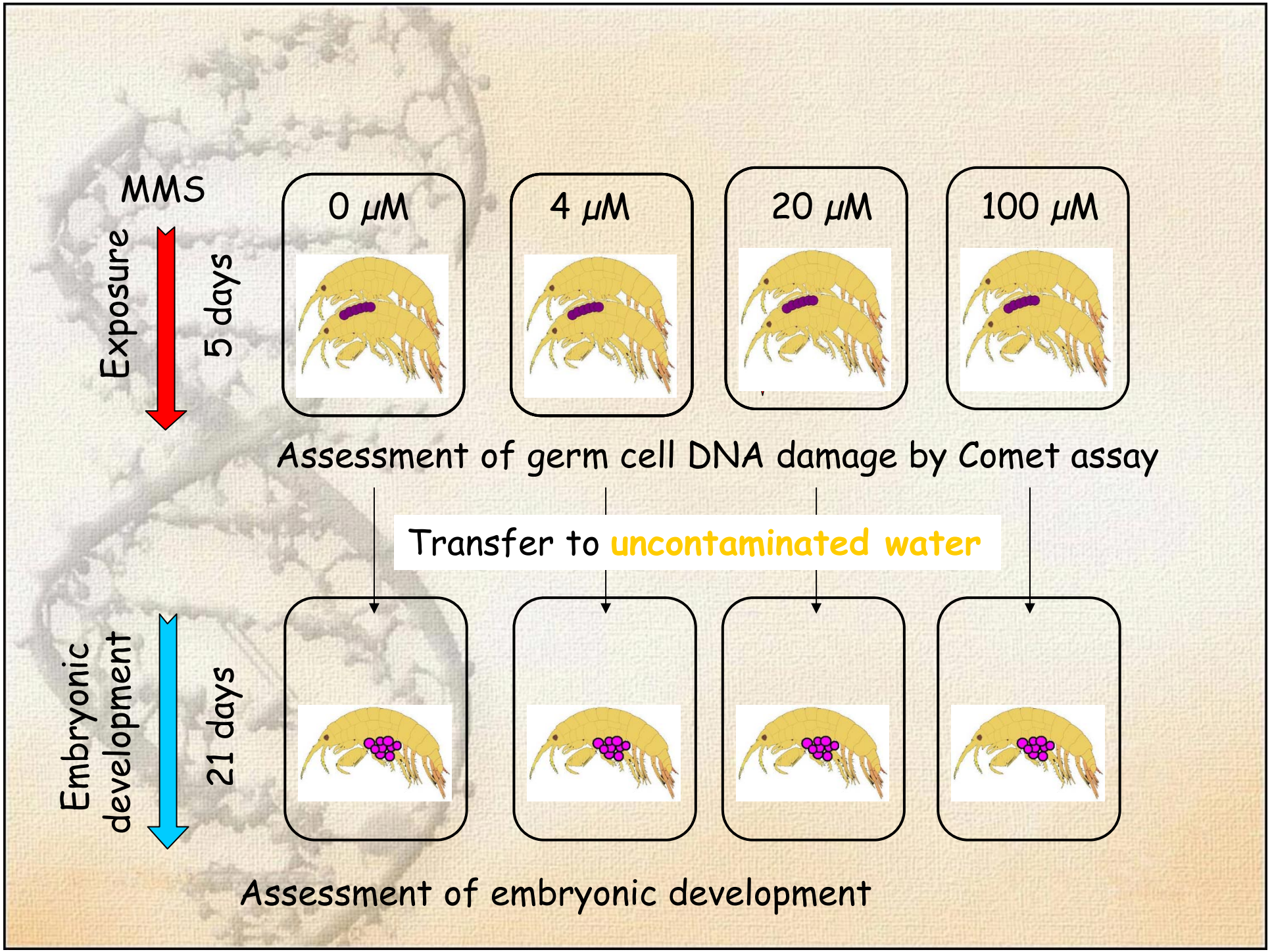
A freshwater crustacean
(Gammarus fossarum)



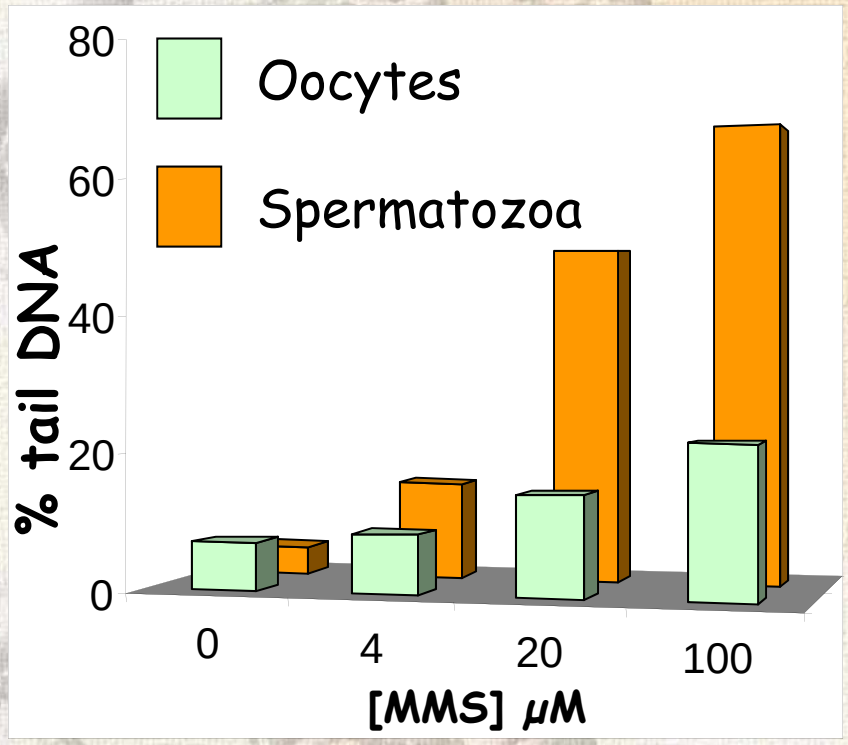
- widespread and abundant in Europe
- sensitive to a large range of chemical stresses
- important food resource for macroinvertebrates, fish, amphibian species and plays a major role in the leaf litter breakdown process and consequently in the entire food web.

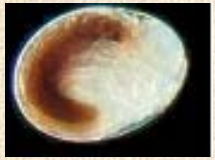


Assessment of germ cell DNA damage by Comet assay

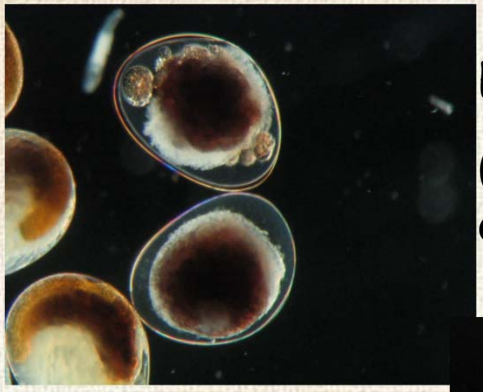

MMS
Exposure
5 days
0 µM
4 µM
20 µM
100 µM
Assessment of germ cell DNA damage by Comet assay
Transfer to uncontaminated water
Embryonic development
21 days
Assessment of embryonic development

After 21 days of development



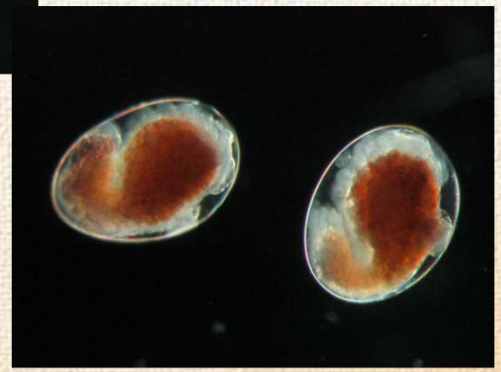


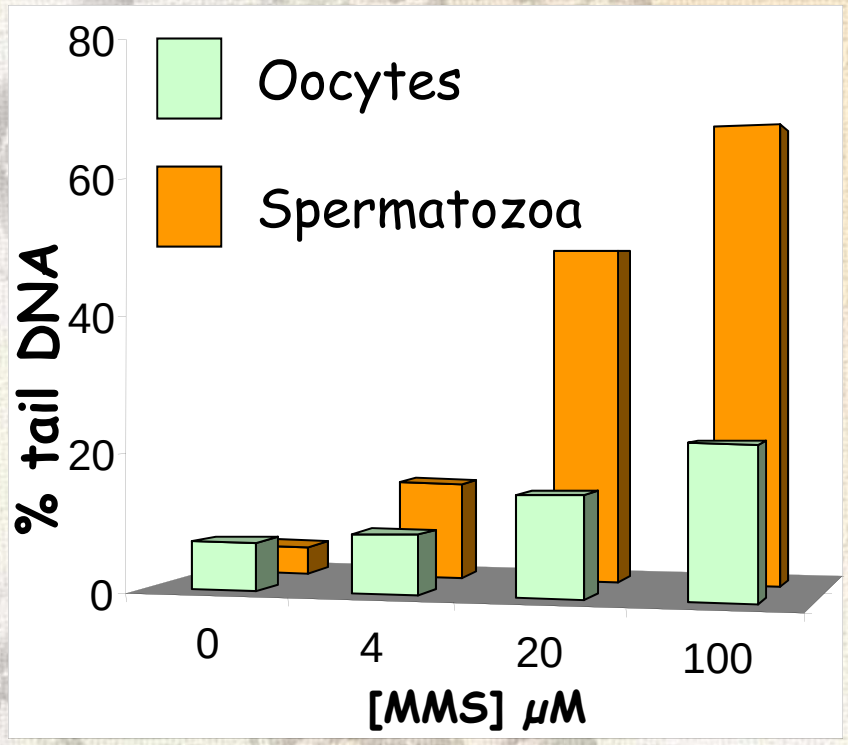
Normal embryo

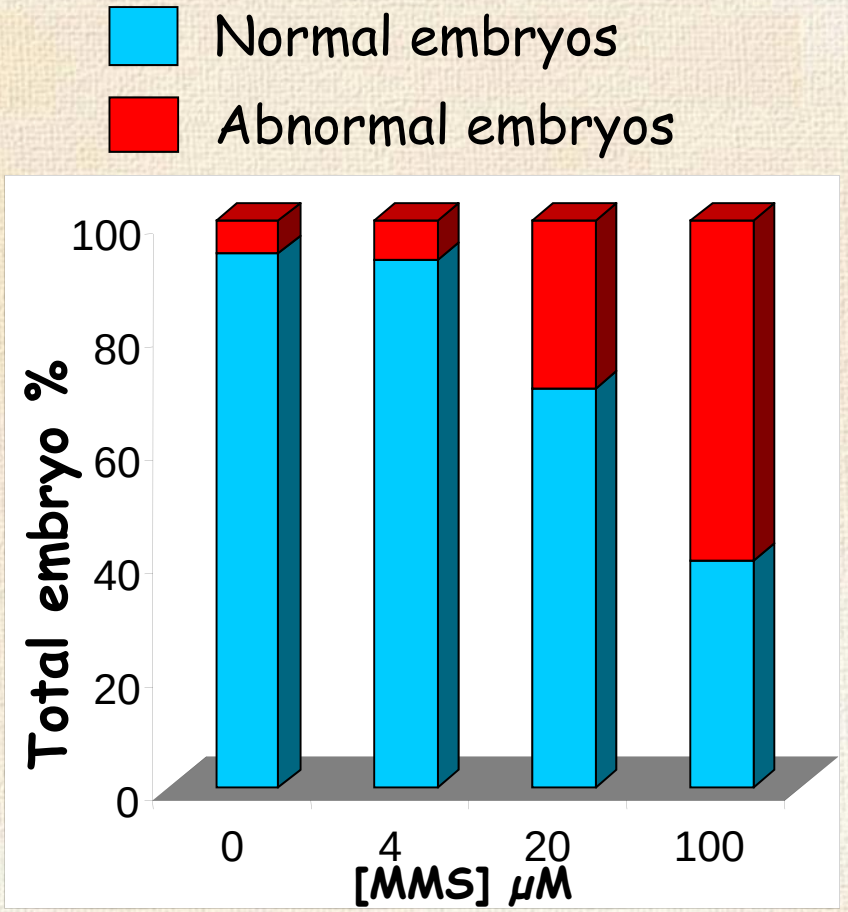


Embryo abnormalities

(abortion, dysmorphy, oedema)







Significant correlation between spermatozoa DNA damage and embryo abnormalities in crustacean (R^2= 0.88)

Conclusions

- spermatozoa: a relevant cell type for assessing genotoxic pressure

Lacaze et al., Environ. Pollut. 2011

-possible transfer of genetic damage from adult to progeny after parental exposure in aquatic species

Devaux et al., Aquat. Toxicol. 2011

Lacaze et al., Environ. Res. 2011

Lacaze et al., Sci. Tot. Environ. 2011

Perspectives

- Abnormality rate increases throughout the offspring development stages ⟶ further studies of integrated responses (F1 reproductive success and F2 survival)

- Oocyte involvement and capacity to repair spermatozoa DNA damage

- Consequences on offspring recruitment and on population sustainability in the field

Field survey on fish populations from contaminated sites
Nase (*Chondrostoma nasus*) and Threespine stickleback (*Gasterosteus aculeatus*)

Action Zone Atelier Bassin du Rhône 2010-2013

Squeletal defect

Mouth defect

Cardiac abnormality

Eye defect

Increase in larval abnormalities in fry stemming from nase caught in polluted areas and that exhibit increased sperm DNA damage

Collaborations :

- **INRA** Thonon
- **Cemagref** Lyon
- **Univ.** Lyon I et Metz
- **INERIS** Verneuil en Halatte

Merci de votre attention