



Acute toxicity of different toxicants to Freshwater Pearl Mussel juveniles (*Margaritifera margaritifera*)

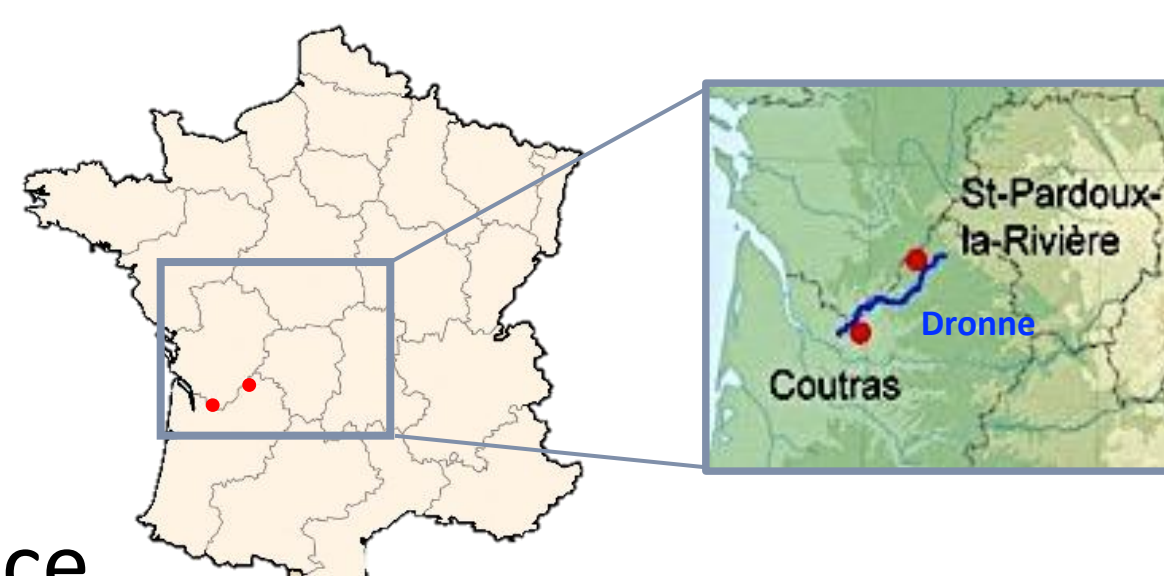
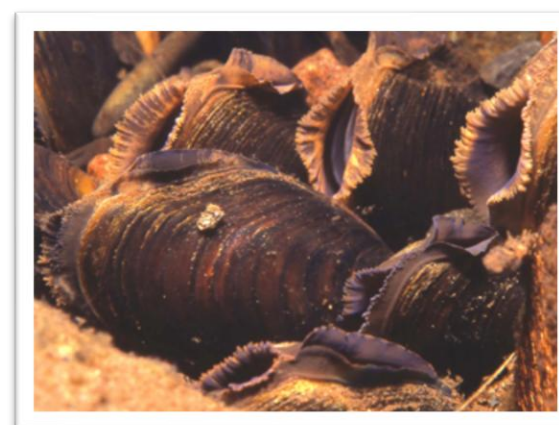
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INTRODUCTION

Margaritifera margaritifera

- Lifetime : up to 80 years
- Filtration rate : 50L/day
- Decline : by 90% in Europe and 99% in France
- 16 000 individuals in the Dronne River (Dordogne-France)



ENDANGERED SPECIES (IUCN)

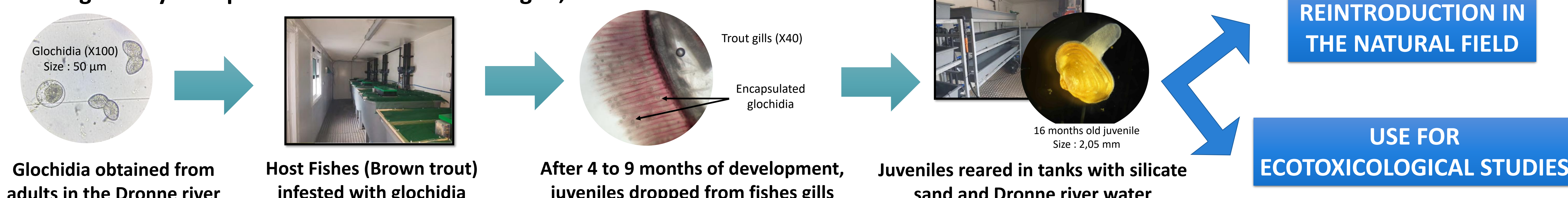
Habitats Directive (Appendix II and V), Berne Convention (Appendix II),
Red List IUCN



Decline causes : Pearl fishing, Habitats degradation, Decline of the host fish, Pollution

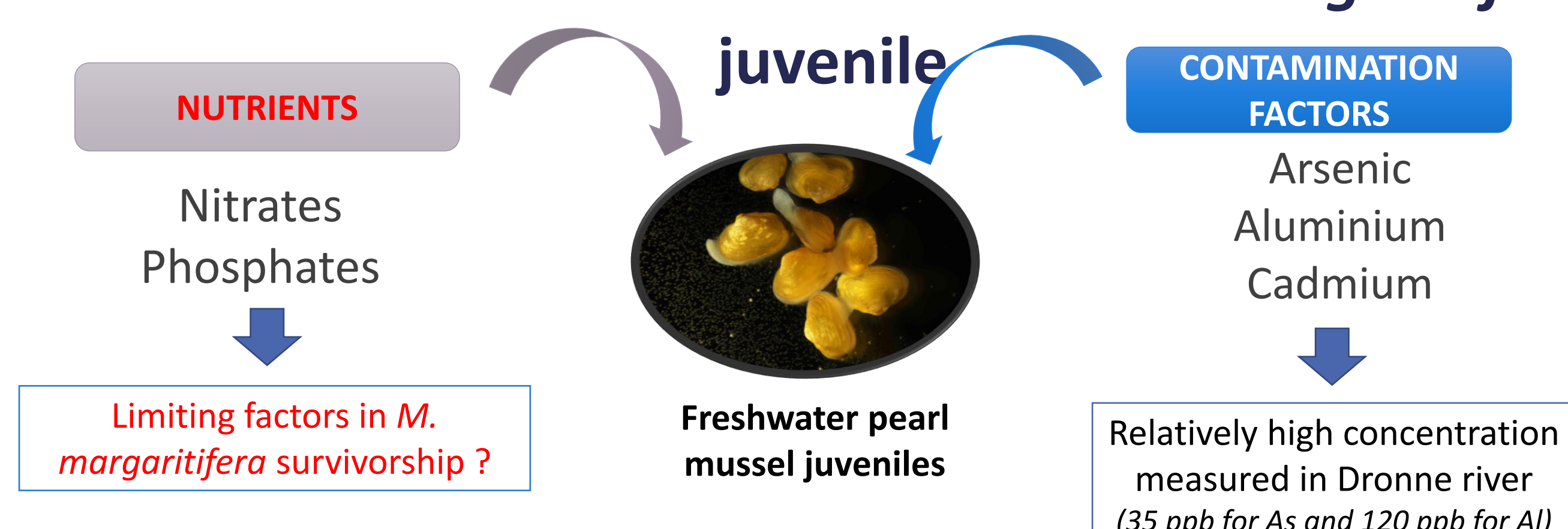
EUROPEAN LIFE PROGRAMME (LIFE13-NAT/FR/000506)

Rearing facility set up in 2016 in Firbeix – Dordogne, FRANCE

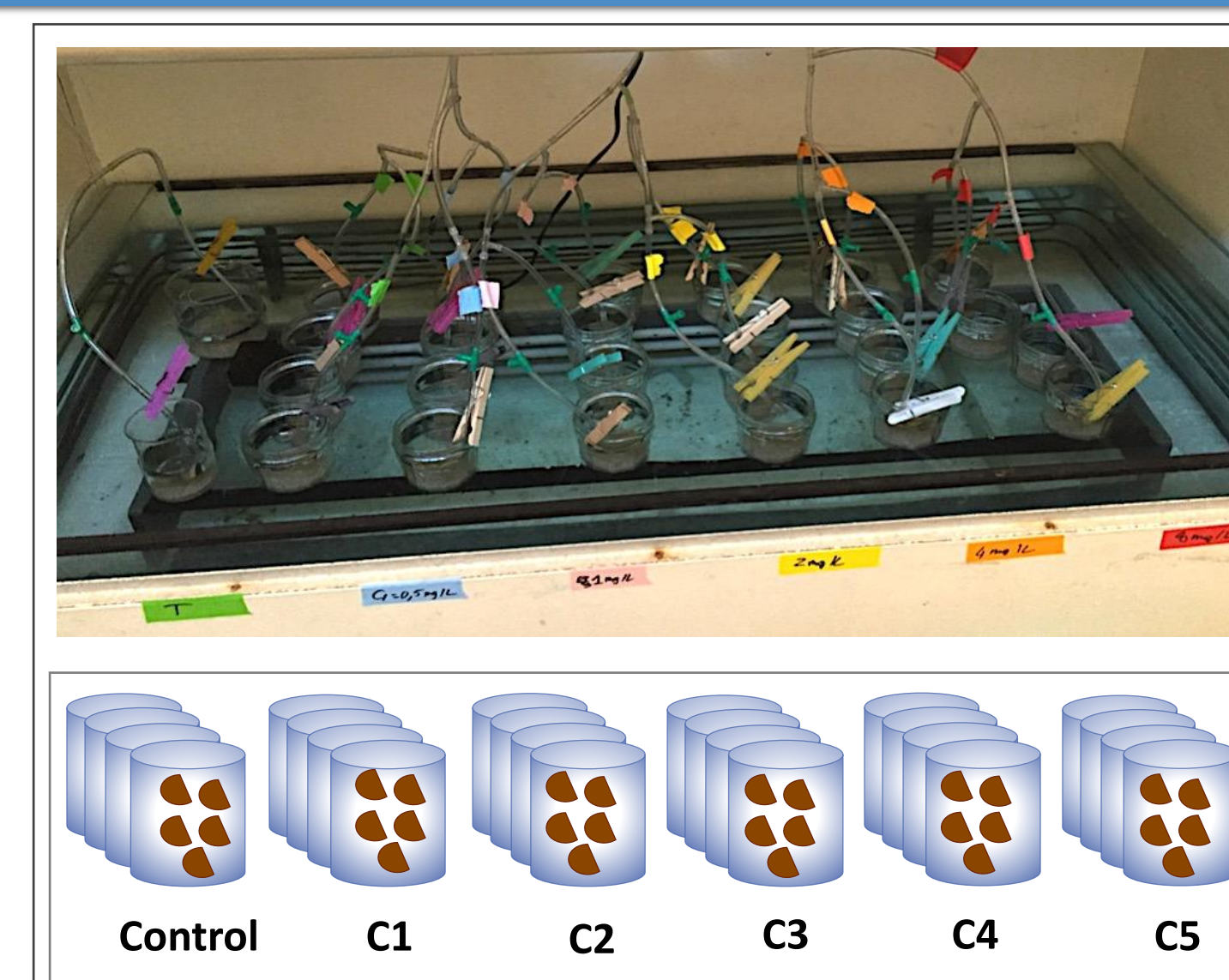


OBJECTIVE OF THE STUDY

Assess median lethal concentration (LC₅₀) of **nutrients** and several toxicants for survival of *M. margaritifera* juvenile



MATERIALS AND METHODS



ACUTE TOXICITY TEST

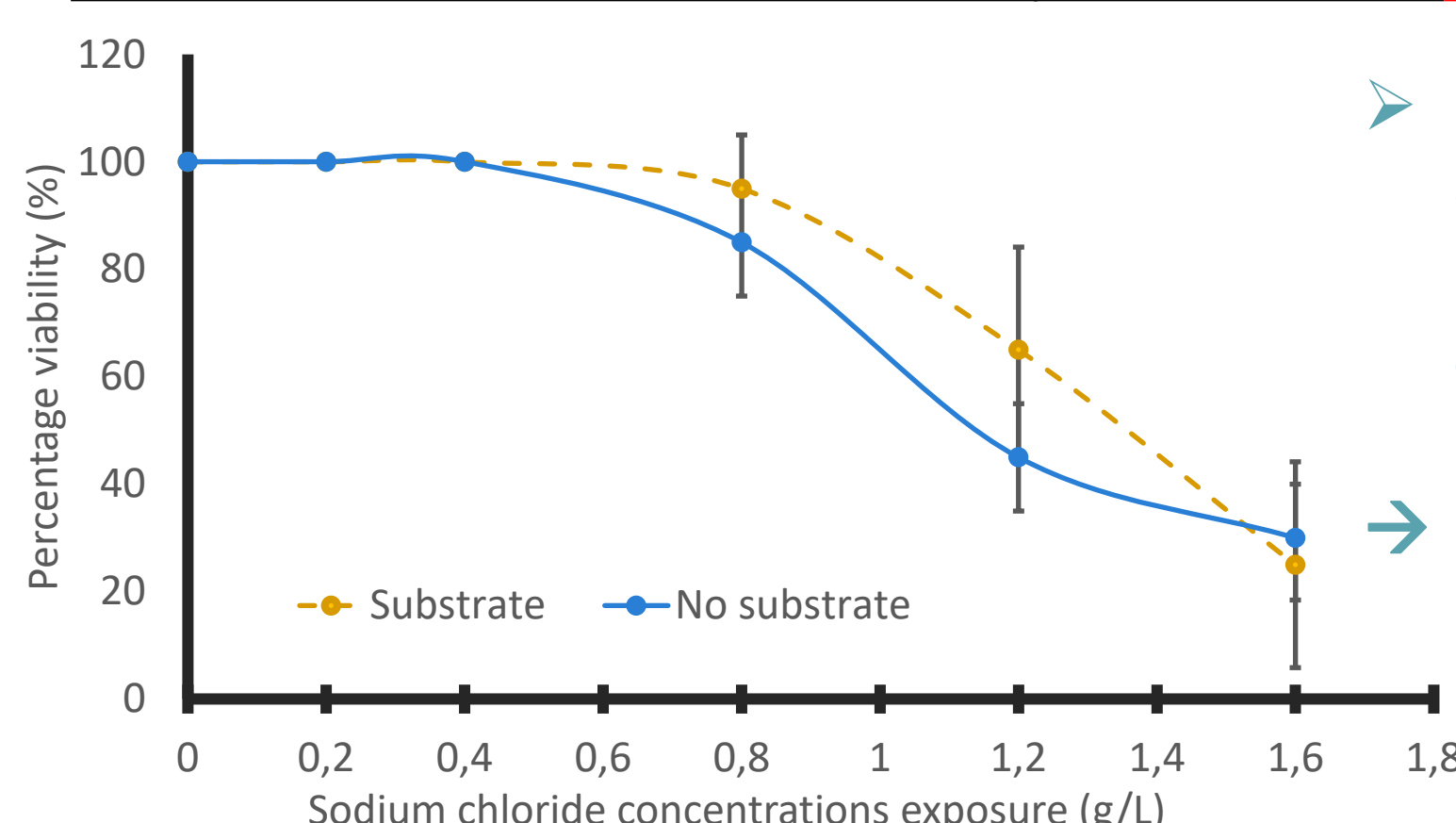
- 20 juveniles/condition
- Temperature : 15°C
- Silicate sand (0,8 – 1,2 mm Ø)
- Test duration : 96h
- Aeration
- Endpoint : survival
- Age of juveniles : 10 to 22 months (after release from the gill)

According to ASTM E 2455 (2006)

RESULTS AND DISCUSSION

VALIDATION OF THE EXPERIMENTAL PROTOCOL

Percentage viability (± SD) of 21 months old *Margaritifera margaritifera*'s juvenile exposed to sodium chloride in 96-h acute toxicity test conducted **with and without substrate**



➤ No statistical difference in viability for each concentration exposure and regarding the presence or the absence of substrate

➔ Validation of our method of exposure

MEDIAN LETHAL CONCENTRATIONS

Contaminants	Age of organisms (months)	Substrate	LC ₅₀ (96h)	Units
NaCl Sodium Chloride	22	No	1,19 (1,11 - 1,28)	g/L
NaCl Sodium Chloride	22	Yes	1,33 (1,24 - 1,42)	g/L
NO ₃ ⁻ Nitrates	13	Yes	> 1000	mg/L
PO ₄ ³⁻ Phosphates	13	Yes	> 6	mg/L
CdCl ₂ Cadmium	17	Yes	> 112	µg/L
CdCl ₂ Cadmium	22	No	> 147	µg/L
CdCl ₂ Cadmium	22	Yes	> 110	µg/L
As ₂ O ₅ Arsenic	18	Yes	> 127	µg/L
AlCl ₃ Aluminium	28	Yes	> 954	µg/L

Median lethal concentrations of several contaminants for survival of *M. margaritifera*'s juvenile
« > » indicates that LC₅₀ is higher than the highest concentration tested

➤ Tolerance to high concentrations of contaminants

AGE RELATED SENSITIVITY

Sodium chloride exposure

	10 months	22 months
48-h LC50 (CI 95%) (g/L)	1.1 (0.87 – 1.33)	1.5 (1.35 – 1.66)

Median lethal concentration of NaCl to survival of *M. margaritifera* juveniles regarding the age of organisms

Cadmium exposure

	17 months	22 months
Cadmium Control	100	100
(µg/L)	5	100
	15	100
	30	100
	60	100
	120	80

Mean survivorship in Cadmium exposure to juveniles of *M. margaritifera* regarding the age of organisms

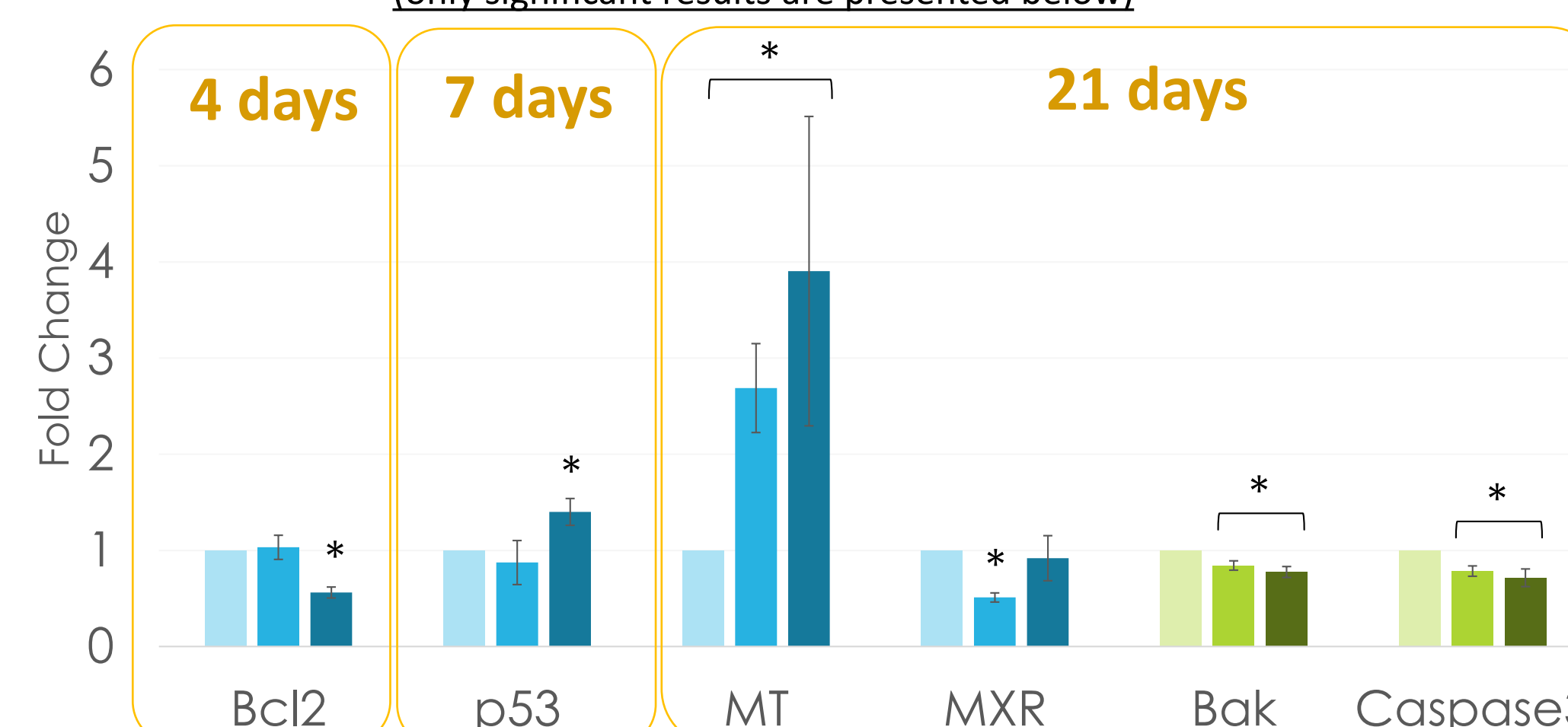
➤ Slight sensitivity for younger juveniles

GENETIC ANALYSES – CHRONIC EXPOSURE (21 days)

Chronic exposure (21 days) of 18 months old juveniles to environmental concentrations of arsenic (As) and cadmium (Cd)

- 21 studied genes → involved in oxidative stress, DNA repair, apoptosis, detoxication, mitochondrial metabolism

Gene expression (compared to control) of 18 months old juveniles after 4, 7 and 21 days exposure to cadmium and arsenic (only significant results are presented below)



High-throughput sequencing of *M. margaritifera*'s transcriptome

Target relevant genes

Quantitative PCR

CADMIUM ARSENIC
Control 2 µg/L 5 µg/L 10 µg/L 35 µg/L

* indicates statistical difference (p < 0.05)

APOPTOSIS
Bcl2, p53, Bak and Caspase3

DETOXICATION
MT, MXR

➤ Gene expression is affected by Cd and As exposure
➔ Genes involved in APOPTOSIS and DETOXICATION

➤ Chronic exposure of As and Cd at non-lethal concentrations might induce effects on apoptosis and detoxication processes
➔ Proteomic studies are needed to confirm those effects

CONCLUSION

- ✓ Juveniles are tolerant to high concentrations of contaminants (in short-term experiment and when studied alone)
- ✓ Younger juveniles are slightly sensitive
- ✓ Genetic analyses show that low concentrations of Cd et As might affect juvenile's biological processes and thus gives us useful data about the toxicity of both metals
- Multistress experiments are needed in order to get closer to the natural environment
- Complementary studies to genetic analyses are needed in order to confirm results obtained from gene expression