

ECOTOXICOLOGICAL STUDIES ON JUVENILE OF *MARGARITIFERA MARGARITIFERA* FOR REINTRODUCTION OF THIS ENDANGERED SPECIES

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EPOC

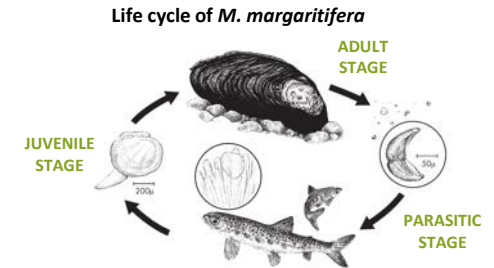


Introduction



Margaritifera margaritifera (Freshwater Pearl Mussel)

- ▶ Lifetime : up to 100 years
- ▶ Complex life cycle
- ▶ Umbrella species
- ▶ **ENDANGERED SPECIES** (RedList IUCN)



Skinner, Young & Hastie (2003)

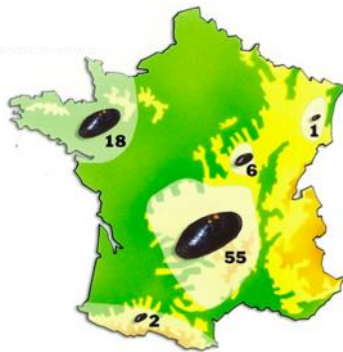


Decline by **90% in Europe** and **99% in France**

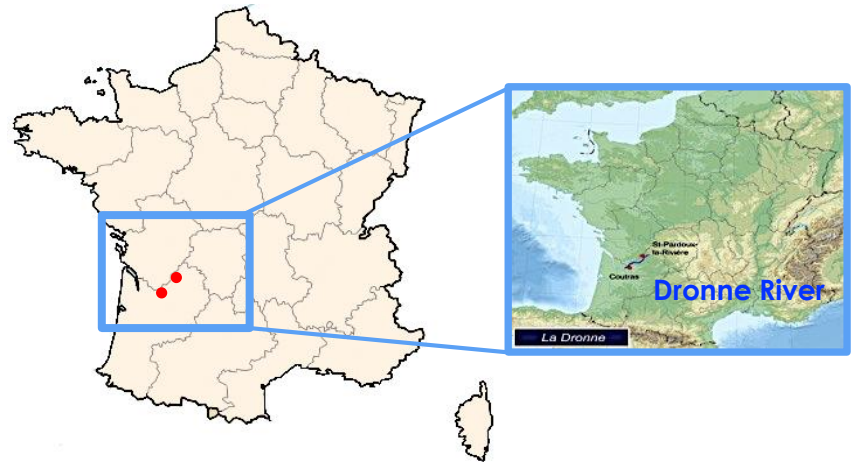
Introduction

Estimation of the French Population : **100 000 individuals**

Found in 82 french rivers



Distribution of *Margaritifera margaritifera* in France (G.Cochet, 2004)



Most important population of France



Dronne river : 16 000 individuals

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

« Preservation of *M.margaritifera* and restoration of river continuity of the Upper Dronne river 2014 – 2020 »

Rearing facility for the production of new freshwater pearl mussel

FIRBEIX - France



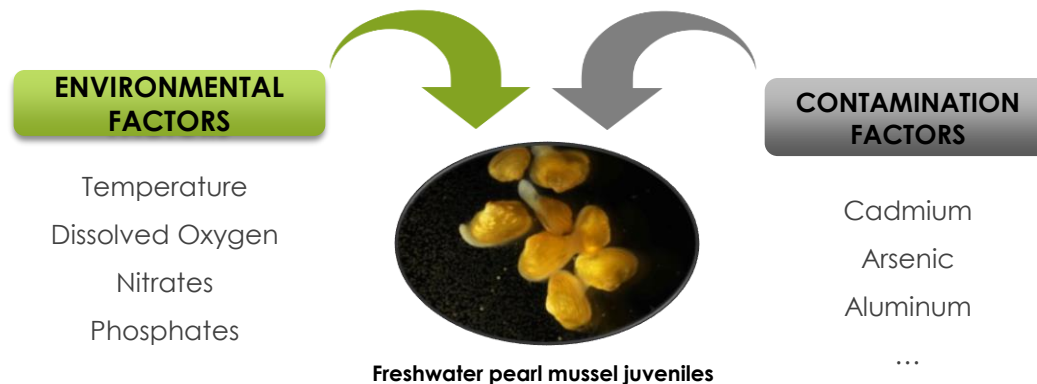
REINTRODUCTION IN THE NATURAL FIELD



USE FOR ECOTOXICOLOGICAL STUDIES

Aim of my work

Study of the sensibility of freshwater pearl mussel juveniles to **environmental** and **contamination factors**



Target reintroduction areas and **improve our knowledge** about *Margaritifera margaritifera*

Aim of my work

ACUTE TOXICITY TEST



Assess LC_{50} values

CHRONIC TOXICITY TEST



Effects on gene expression
and juveniles behaviour

MULTIFACTORIAL TEST



Synergistic or antagonistic
effects

Aim of my work

ACUTE TOXICITY TEST



Assess LC₅₀ values

- ✓ **Nitrates**
- ✓ **Phosphates**
- ✓ **Cadmium**
- ✓ **Arsenic**

CHRONIC TOXICITY TEST



Effects on gene expression and juveniles behaviour

- ✓ **Arsenic**
- ✓ **Cadmium**

MULTIFACTORIAL TEST



Synergistic or antagonistic effects

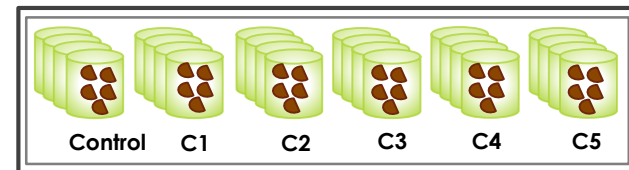
Materials and methods

ACUTE TOXICITY TEST

EXPERIMENTAL CONDITIONS

- 20 juveniles/treatment
- Temperature : 15°-16°C
- Sediment (0,8 – 1,2 mm Ø)
- Dronne river water
- Aeration
- Endpoint : survival
- Static non-renewal test
- **Age of juveniles : 13 to 16 months**

Test duration : 96 hours



According to ASTM E 2455 (2006) with modifications

Materials and methods

CHRONIC TOXICITY TEST

EXPERIMENTAL CONDITIONS

- 30 juveniles/treatment
- Temperature : 15°-16°C
- Sediment (0,8 – 1,2 mm Ø)
- Aeration
- Fed with freshwater algal mixture
- Endpoints : survival, genetic analysis
- Static-renewal test (50% twice a week)
- **Age of juveniles : 16 months**

Test duration : 21 days



Materials and methods

CHRONIC TOXICITY TEST

EXPERIMENTAL CONDITIONS

- 30 juveniles/treatment
- Temperature : 15°-16°C
- Sediment (0,8 – 1,2 mm Ø)
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GENETIC ANALYSIS

Quantitative PCR

(high-throughput sequencing of *M.margaritifera*'s transcriptome)

- Oxidative stress
- Mitochondrial metabolism
- Detoxication
- Apoptosis
- DNA repair
- Translocation

Reference gene : RPL7 (ribosomal protein L7)

Results

ACUTE TOXICITY TEST

Toxicant concentrations studied

		Nominal concentrations (mg/L)	Mesured concentrations ($\pm$ SD) (mg/L)			Nominal concentrations (μ g/L)	Mesured concentrations ($\pm$ SD) (μ g/L)
Nitrates	Control	0	4	Arsenic	Control	0	4,89 $\pm$ 0,70
	C1	128	130 $\pm$ 0,4		C1	5	9,84 $\pm$ 0,85
	C2	256	246 $\pm$ 1,4		C2	15	21,65 $\pm$ 2,69
	C3	512	493 $\pm$ 8,3		C3	30	34,64 $\pm$ 2,62
	C4	1024	1026 $\pm$ 51,5		C4	60	68,46 $\pm$ 12,83
	C5	2048	2290 $\pm$ 85,7		C5	120	127,25 $\pm$ 22,76
Phosphates	Control	0	0,02 $\pm$ 0,01	Cadmium	Control	0	0,03 $\pm$ 0,04
	C1	0,2	0,08 $\pm$ 0,11		C1	5	3,95 $\pm$ 3,59
	C2	0,6	0,32 $\pm$ 0,22		C2	15	11,56 $\pm$ 11,76
	C3	1,6	1,12 $\pm$ 0,29		C3	30	23,06 $\pm$ 24,48
	C4	3	2,30 $\pm$ 0,31		C4	60	51,96 $\pm$ 45,42
	C5	6	5,01 $\pm$ 0,33		C5	120	112,55 $\pm$ 80,55

Results

ACUTE TOXICITY TEST

LC₅₀ values (96h) for Nitrates, Phosphates, Cadmium and Arsenic

Toxicants	LC ₅₀ values (96h)	Mean survival at highest concentration (96h) (%)
NO ₃ ⁻ (mg/L)	1000 -1500	0
PO ₄ ³⁻ (mg/L)	> 5,01	100
Cadmium (µg/L)	> 112,1	80
Arsenic (µg/L)	> 127	93,75

Results

ACUTE TOXICITY TEST

LC₅₀ values (96h) for Nitrates, Phosphates, Cadmium and Arsenic

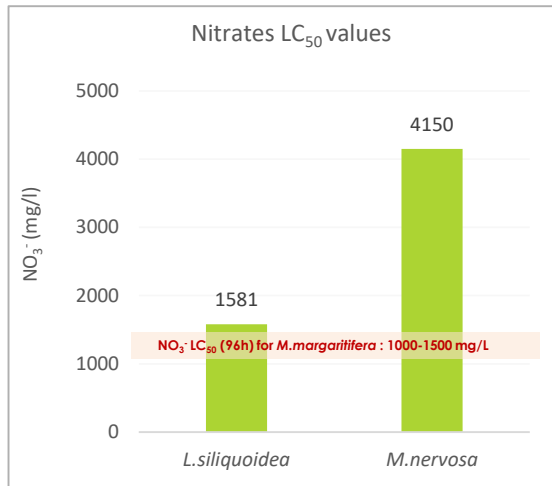
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Arsenic (µg/L)	> 127	93,75

Two highest concentrations for nitrates (mg/l)	Mean mortality after 96h (%)
1024	80
2048	100

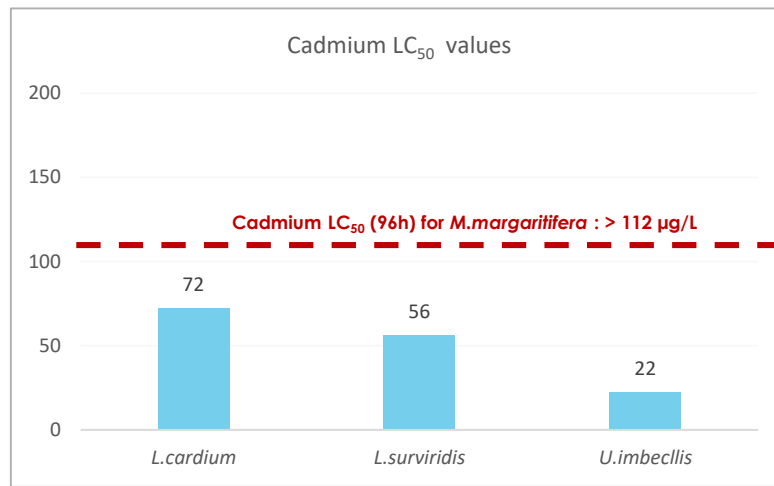
Results

ACUTE TOXICITY TEST

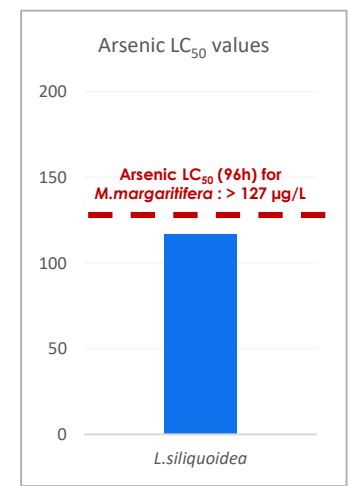
Comparison with the LC₅₀ values of other freshwater mussel



(D.J.Soucek 2012)



(N.Wang 2010)



(N.Wang 2017)

Age of juveniles

- *M. margaritifera* : 16 months
- All others mussels : <5 days

Results

CHRONIC TOXICITY TEST

Toxicant concentrations tested

		Nominal concentrations ($\mu\text{g/L}$)	Mesured concentrations ($\pm$ SD) ($\mu\text{g/L}$)
As	Control	0	4,71 $\pm$ 0,47
	C1	10	15,20 $\pm$ 2,05
	C2	35	43,21 $\pm$ 6,39
Cd	Control	0	0,04 $\pm$ 0,04
	C1	2	2,27 $\pm$ 1,52
	C2	5	5,29 $\pm$ 3,35

Results

CHRONIC TOXICITY TEST

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- Maximum arsenic concentration measured in the Dronne River (2015) : 35 $\mu\text{g/L}$

Results

CHRONIC TOXICITY TEST

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- Maximum arsenic concentration measured in the Dronne River (2015) : 35 $\mu\text{g/L}$
- Compare gene expression of juveniles and adults from the same population (Baudrimont, 2012)

Results

CHRONIC TOXICITY TEST

Mean survival (%) at 0,4,7 and 21 days

		d0 (n=90)	d4 (n=60)	d7 (n=30)	d21 (n=30)
Cd	Control	100	100	100	100
	C1	100	98,33	100	100
	C2	100	100	100	100
As	Control	100	100	100	100
	C1	100	100	100	100
	C2	100	100	93,33	100

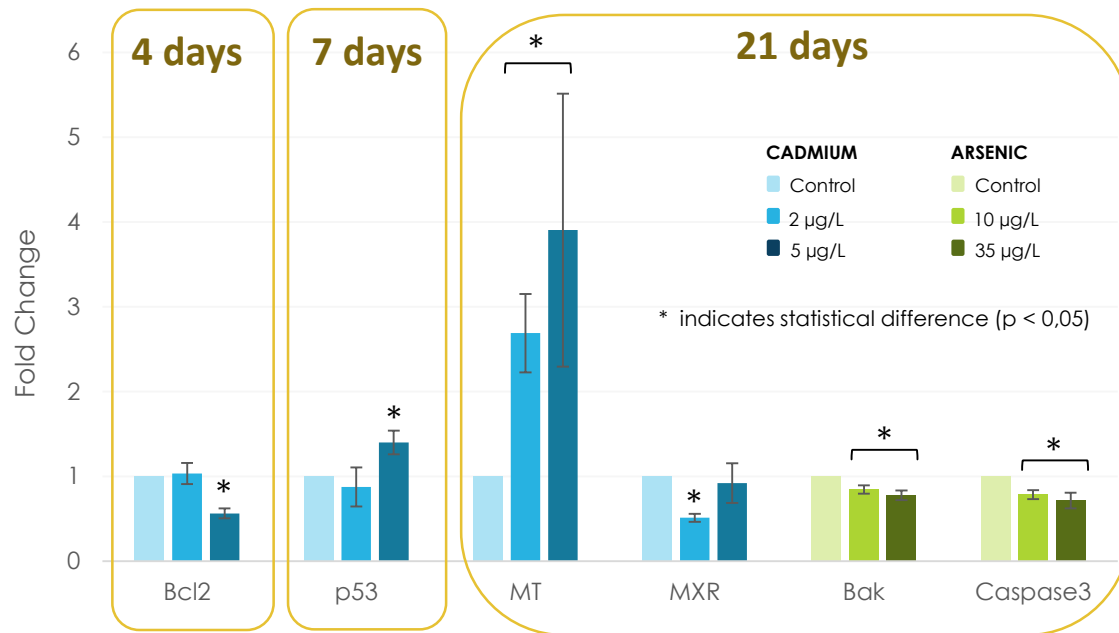
n = total number of organisms per treatment

→ Viability is not affected during the test

Results

CHRONIC TOXICITY TEST

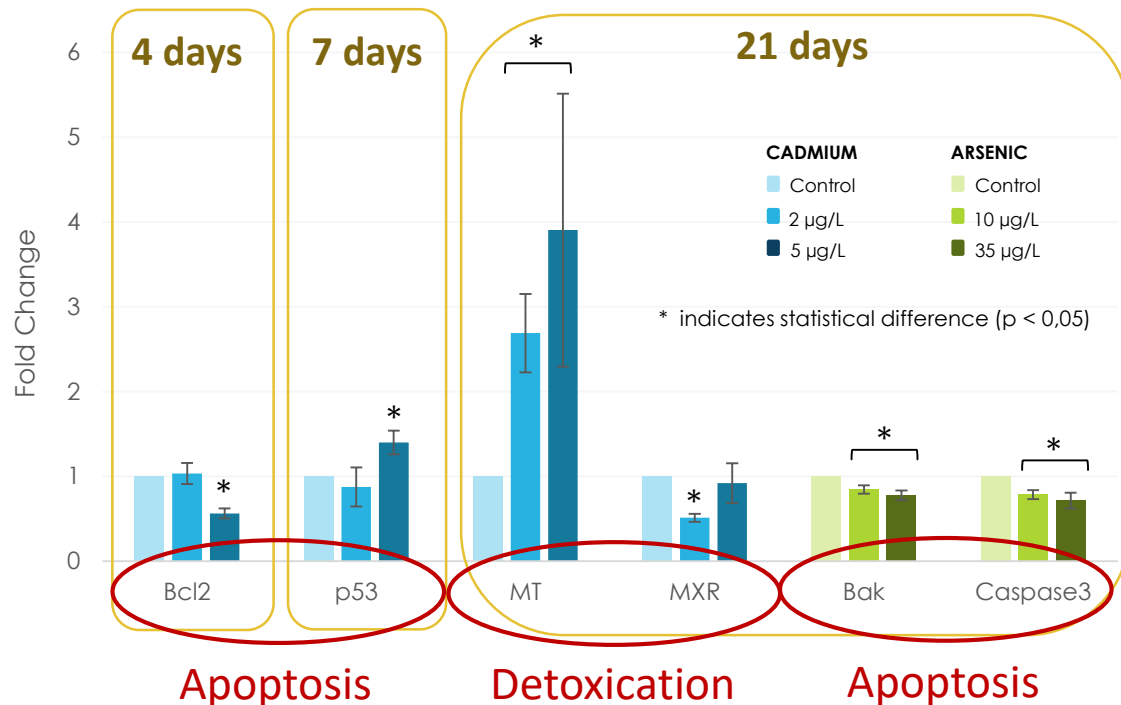
Gene expression (compared to control) after 4, 7 and 21 days exposure to Cadmium and Arsenic



Results

CHRONIC TOXICITY TEST

Gene expression (compared to control) after 4, 7 and 21 days exposure to Cadmium and Arsenic



Effect of chronic exposure to cadmium

- 4 and 7 days : genes involved in **apoptosis**
- 21 days : genes involved in **detoxication**

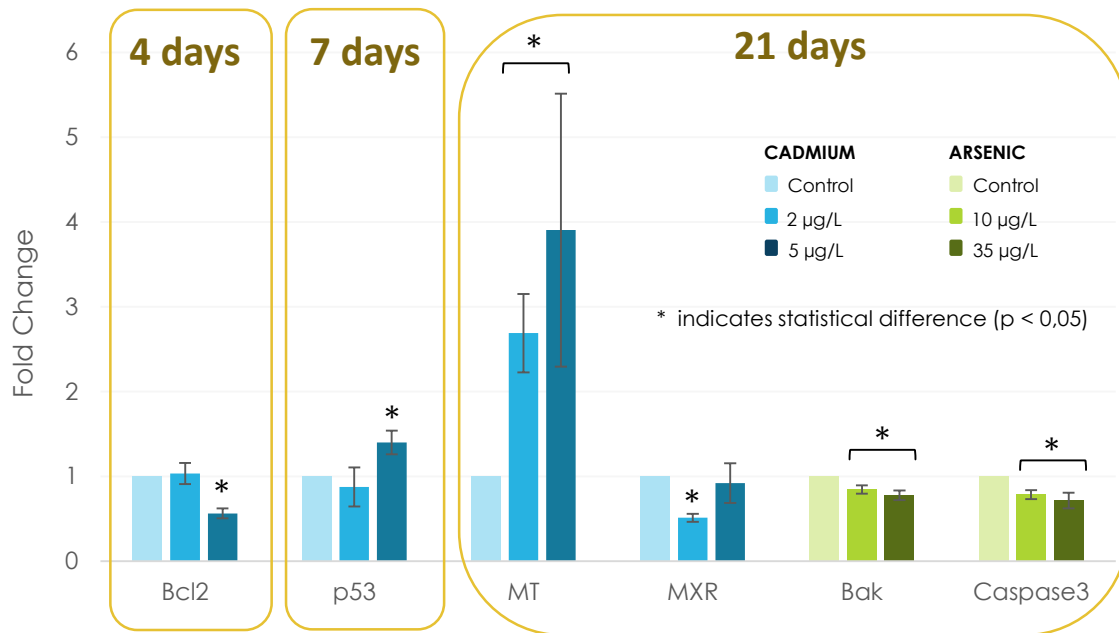
Effect of chronic exposure to arsenic

- 21 days : genes involved in **apoptosis**

Results

CHRONIC TOXICITY TEST

Gene expression (compared to control) after 4, 7 and 21 days exposure to Cadmium and Arsenic



Effect of chronic exposure to cadmium

- 4 and 7 days : genes involved in **apoptosis**
- 21 days : genes involved in **detoxication**

Effect of chronic exposure to arsenic

- 21 days : genes involved in **apoptosis**



Chronic exposure of juveniles to cadmium and arsenic at non-lethal concentrations might **induce effects on apoptosis process and detoxication**

Results

CHRONIC TOXICITY TEST

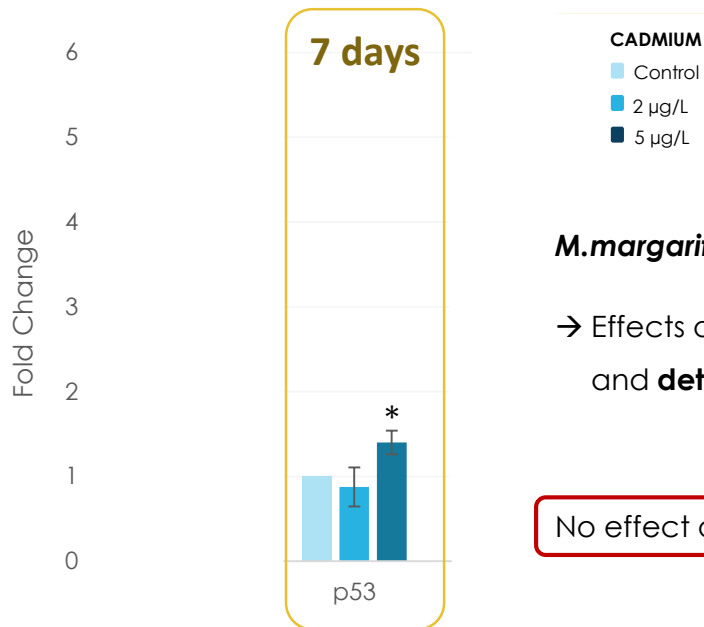
***M.margaritifera* adults after 7 days of exposure to 2 and 5 µg/L Cadmium**

→ Effects on genes involved in **oxydative stress**, **mitochondrial metabolism** and **detoxication**

Results

CHRONIC TOXICITY TEST

Gene expression (compared to control) of juveniles after 7 days of exposure to Cadmium



***M. margaritifera* adults after 7 days of exposure to 2 and 5 µg/L Cadmium**

→ Effects on genes involved in **oxydative stress**, **mitochondrial metabolism** and **detoxication**

No effect on the same genes for juveniles

Conclusion

- ✓ Juveniles of *M.margaritifera* **are tolerant** to high concentrations of **nitrates, phosphates, cadmium and arsenic**
 - Target reintroduction areas for captive freshwater pearl mussel

- ✓ Chronic exposure to Arsenic and Cadmium **induce modification in the expression of genes involved in apoptosis and detoxication**
 - Gene expression depends on the age (as seen for *M.margaritifera* adults)
 - Further studies on protein production are necessary to confirm results on gene expression

THANK YOU FOR YOUR ATTENTION !



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