



Ecotoxicological study of the impacts of metals contamination on the pearl mussel *Margaritifera margaritifera* in the Dronne river, France

Magalie Baudrimont

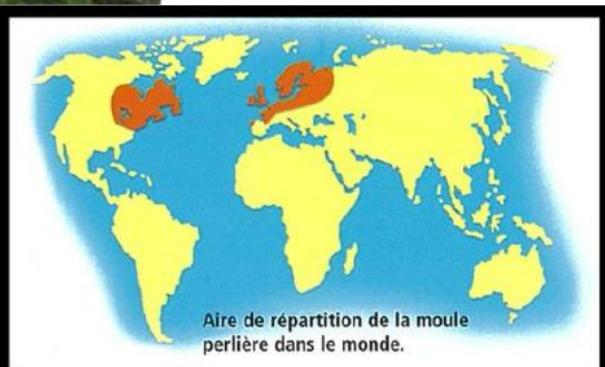
Anthony Bertucci, Fabien Pierron, Patrice Gonzalez, Julien Thébault, Julie Bellec, Joan Vieira, Christophe Klopp, Pierre-Yves Gourves, Nathalie Mesmer-Dudons, Bruno Etcheverria, Alexia Legeay



European project LIFE13-NAT_FR_000506 (2014-2020)
Préservation de *Margaritifera margaritifera* et restauration de la
continuité écologique de la Haute Dronne



The pearl mussel *M. margaritifera*



Mollusk bivalve unionidae

Geographical repartition: on the North Atlantic sides of America and Europe, from Spain to Scandinavia (corresponds to repartition area of atlantic salmon).

Habitat: clean flowing waters, gravel / sandy bottoms

Lifespan: near 150 years in Scandinavia, around 100 years in France. Sexual maturity between 10 and 20 years.

Particularity: produces pearls with a frequency of 1 pearl for 1000 individuals.



An endangered species...



Endangered species: decline of its populations up to 90% in Europe, near 99% in France during XXth century

- Pearls exploitation until the middle of the XXth century → Marie de Médicis dress with 32 000 pearls!!!
- Degradation of habitats quality, climate change, sedimentation, nutrients enrichment (agriculture), discharges, landfill sites...

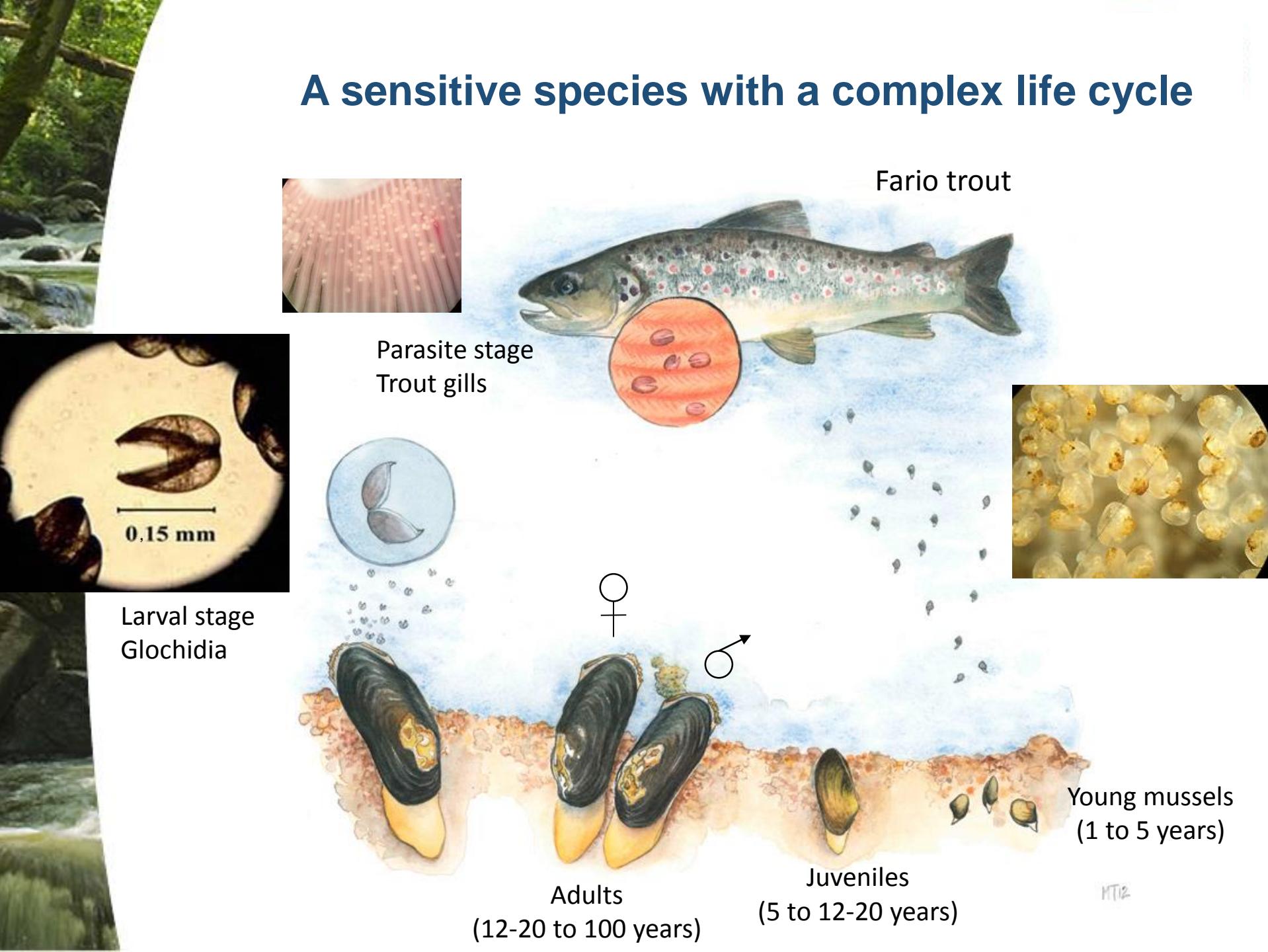


Protection status:

- **IUCN red list from 1996**
- Directive Habitat (annexes II and V)
- Convention de Berne (annexe III)
- PNA 2012-2017
- PRA Limousin (LNE)

**Upper Dronne: 16,000 individuals
≈ 15 % of national population**

A sensitive species with a complex life cycle



First ecotoxicological study on the Dronne river

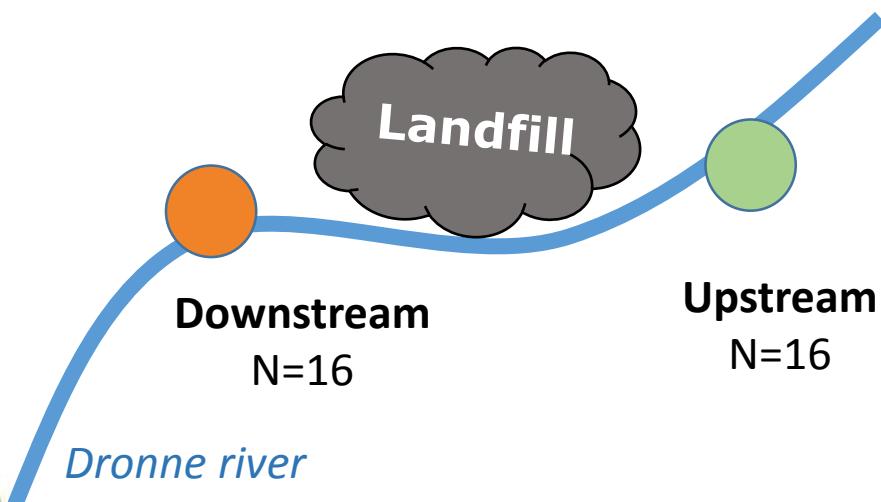
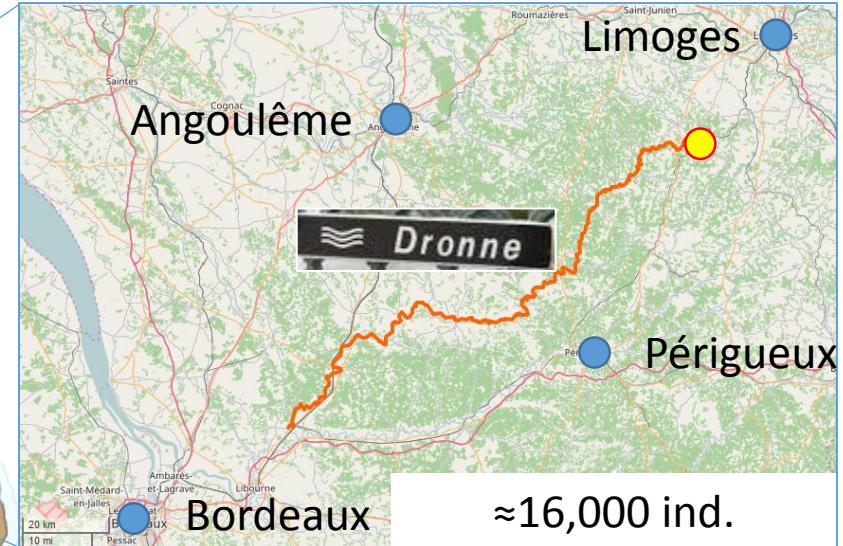
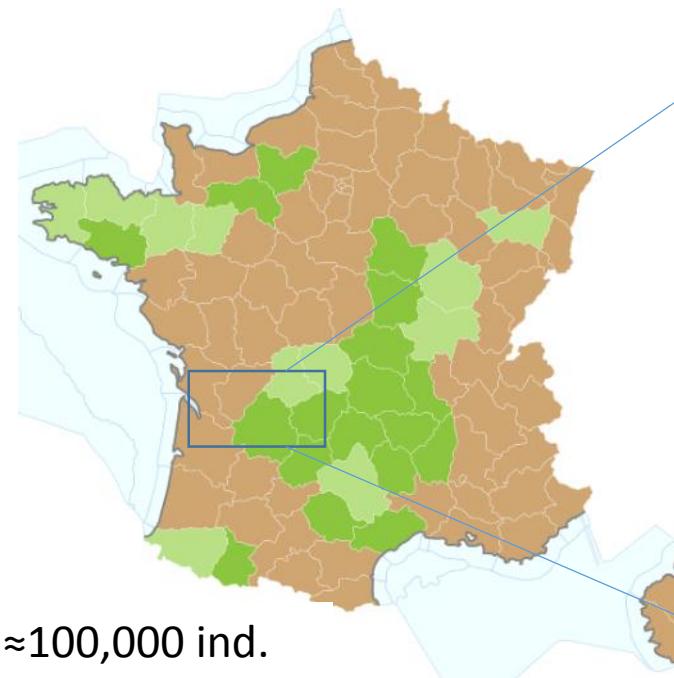
- **Dronne river:** slightly polluted, but activities on its catchment basin led to a progressive degradation of its quality.
- **Landfill at St Saud Lacoussière,** just above a *M. margaritifera* pavement (1,000 to 1,500 individuals)



Impact of micropollutants on mussels?

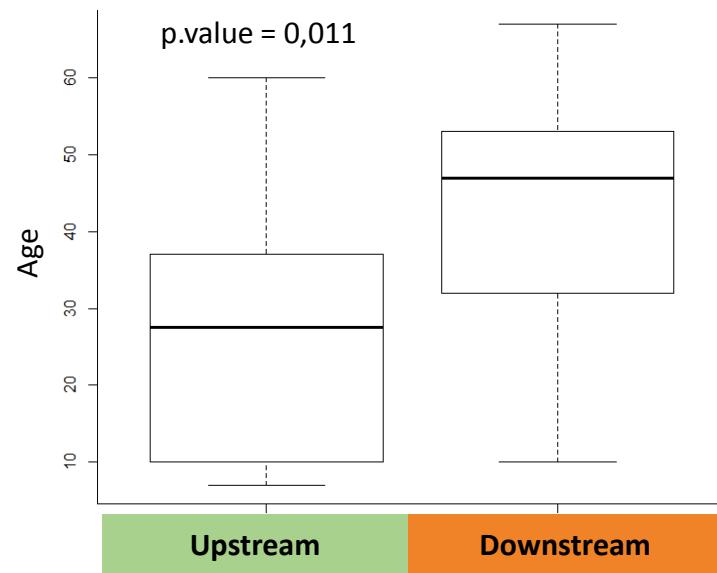
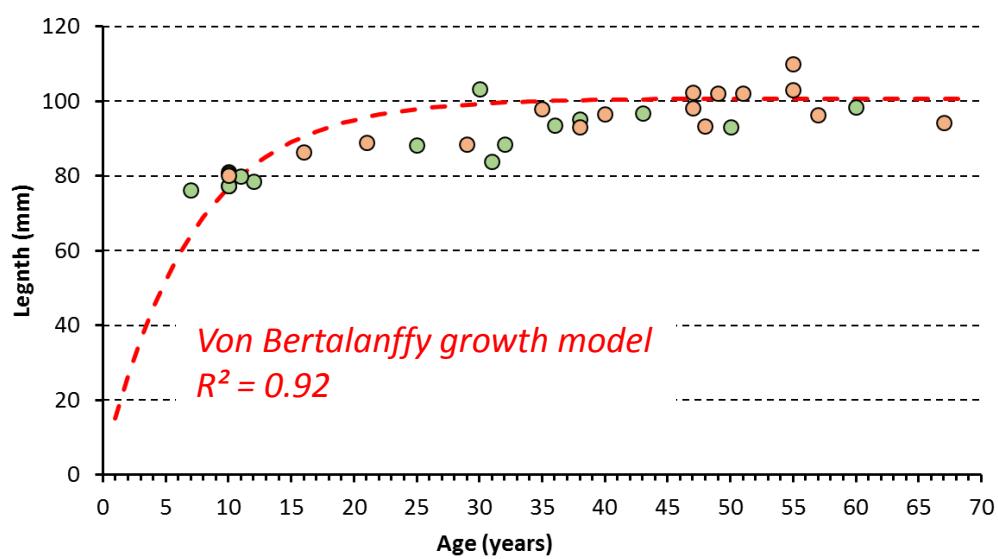
**Autorization of collection for 113 individuals in the Dronne river
by the Ministry of Environment in 2008**

METHODS



- 1. Shell:** sclerochronology
- 2. Digestive Gland:** Metals
- 3. Kidney:** RNA-seq

RESULTS: Age determination



Age was not directly assessable *in situ*.

Size is not a good age proxy.

Mussels that were sampled downstream were actually older.

RESULTS: Metal contamination

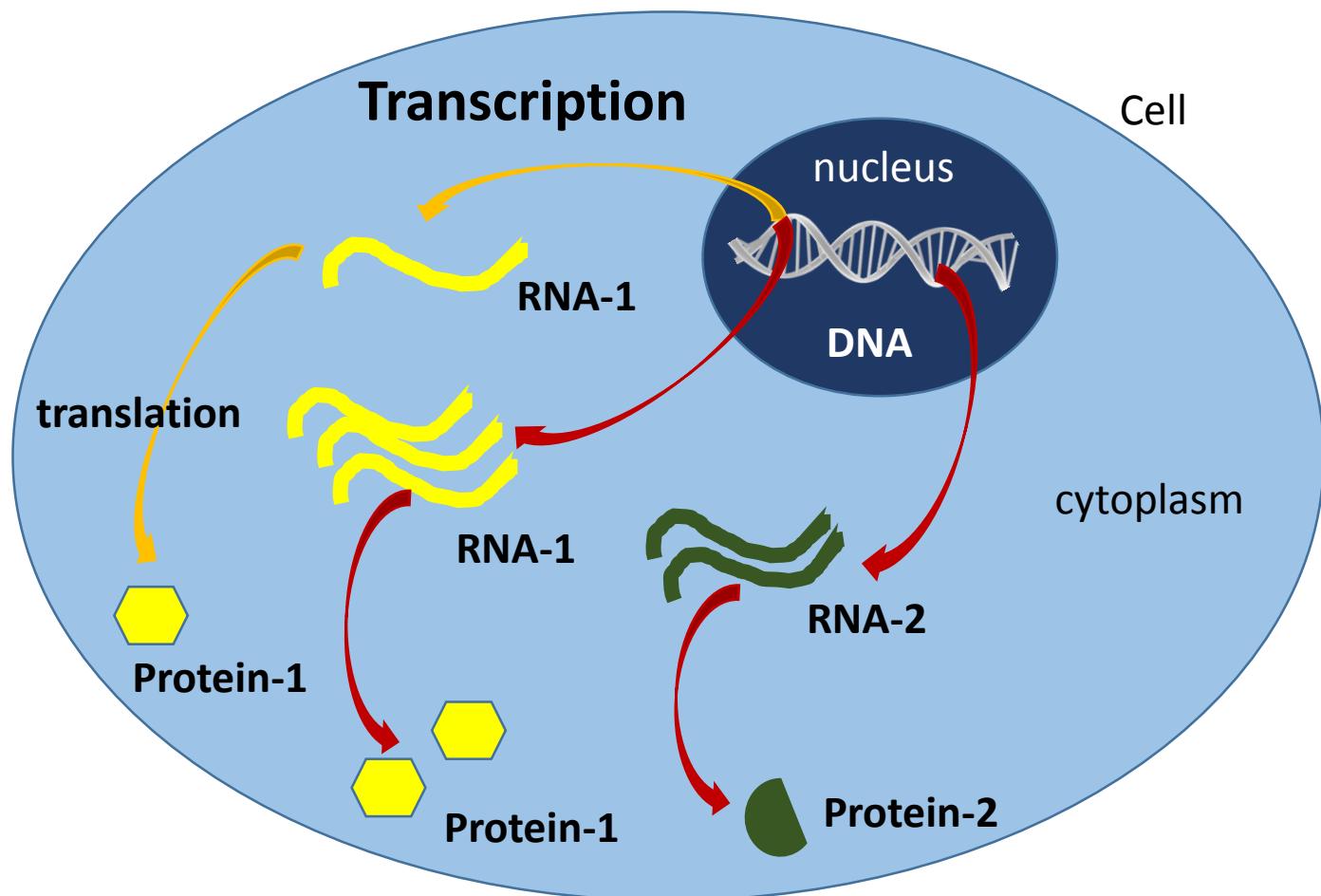


		Upstream	Downstream
Physico-chemistry	Temp. (°C)	6.10	6.00
	pH	7.36	7.26
	Cond. (µS)	85	84
	O ₂ (mg/L)	11.72	12.05
Biometry	Length (mm)	87.03 ± 2.12	95.88 ± 1.81 (**)
	Age (y)	25.94 ± 4.03	41.56 ± 3.85 (**)
	CI	57.29 ± 4.06	42.69 ± 2.3 (**)
Metals (µg/g dry weight)	As	6.48 ± 0.38	8.49 ± 0.56 (**)
	Cd	1.36 ± 0.11	3.58 ± 0.68 (**)
	Co	0.98 ± 0.09	1.7 ± 0.12 (***)
	Cr	2.58 ± 0.46	5.01 ± 0.83 (**)
	Cu	12.22 ± 1.08	10.66 ± 1.43
	Mn	947.34 ± 190.01	1545.88 ± 222.88
	Ni	0.58 ± 0.05	0.9 ± 0.1 (*)
	Pb	1.46 ± 0.42	1.82 ± 0.43
	Zn	75.26 ± 1.72	81.78 ± 2.56 (*)

* 0.05 ; ** 0.01 ; *** 0.001

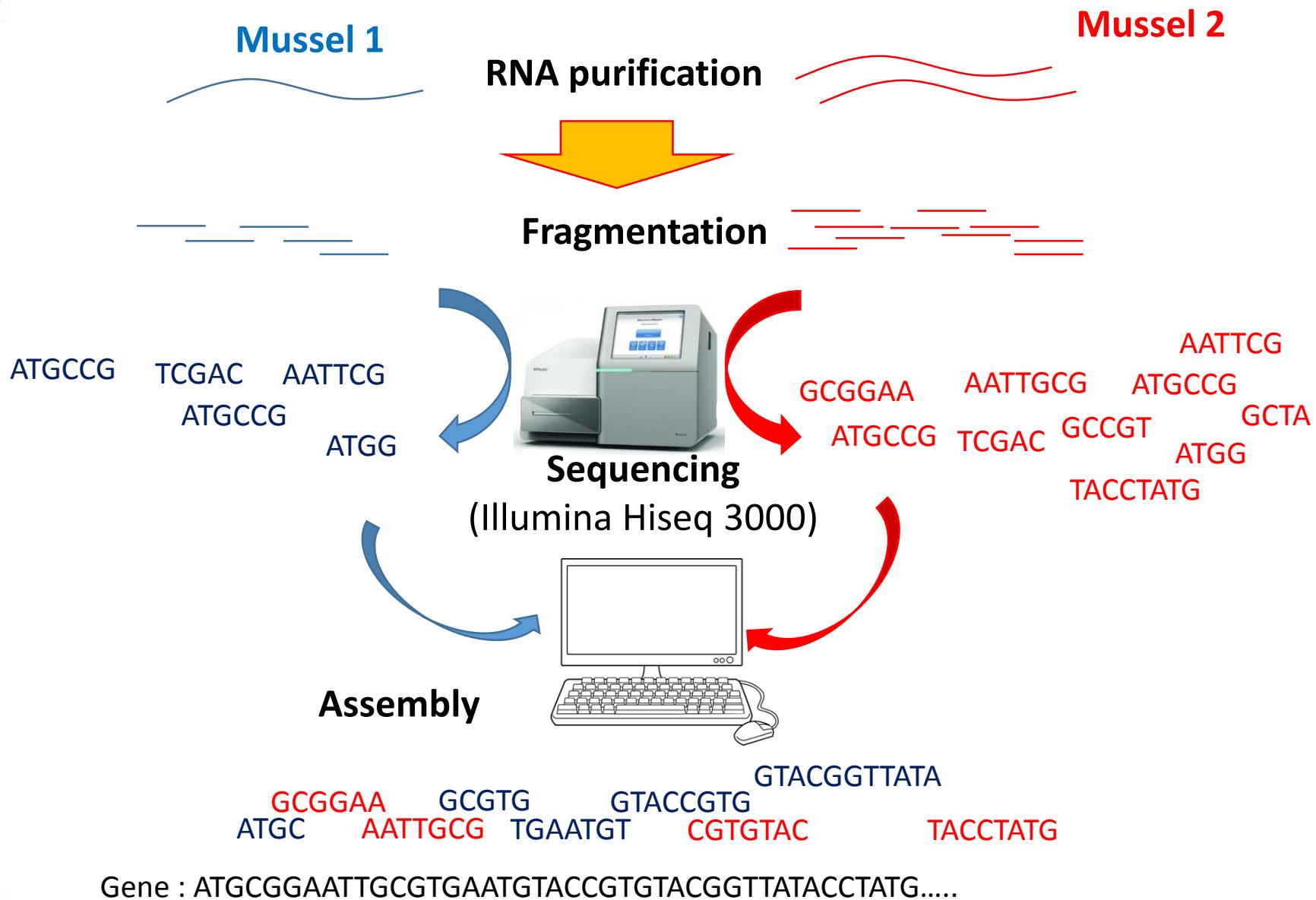
Downstream mussels were more contaminated.
What is the biological outcome ?

Transcriptomic approach



Transcriptome = expressed genes

Principle of RNAseq



RESULTS: Transcriptome assembly



3 Billion RNA fragments → 51,392 transcripts
→ 22 288 known (43%)



<http://ngspipelines2.toulouse.inra.fr:9007/>

The screenshot shows the NGS Pipelines interface. At the top, there's a navigation bar with links for Home, Contigs, Variants, Download, and Help. A sub-navigation bar for 'M. M. - LifeMarga' is active, showing 'Margaritifera Margaritifera - LifeMarga The Project Uses 155.98 Gb On The Harddrive'. Below this, a thumbnail image of a mussel shell is labeled 'Transcriptome of Margaritifera margaritifera'. On the left, there are tabs for 'Available Applications Logs' and 'Contigs list of analyses'. The 'Contigs list of analyses' tab is selected, displaying a table with 10 entries. The columns are 'Name', 'Software', and 'Comment'. The table includes rows for tRNAscan-SE, RNAmmer, blast, and Interproscan. At the bottom, there are sections for 'Links' (GenoToul Bioinfo platform, Sigenae platform, INRA) and 'About NGS Pipelines' (a brief description of the platform). A copyright notice at the very bottom states: 'Copyright © 2012, INRA | Designed by GenoToul Bioinfo and Sigenae teams | Optimized for' followed by two small circular icons.

NCBI GEO : GSE94542

The screenshot shows the NCBI GEO Accession viewer for series GSE94542. The top header includes the NCBI logo and links for HOME, SEARCH, SITE MAP, GEO Publications, FAQ, MIAME, Email GEO, Contact, and Sign Out. The main content area has a title 'Series GSE94542' with a 'PRIVATE UNTIL MAR 01, 2018' status. It provides detailed information about the study: Title (Transcriptomic responses of the endangered freshwater mussel Margaritifera margaritifera to in situ trace metals contamination), Organism (Margaritifera margaritifera), Experiment type (Expression profiling by high throughput sequencing), and Summary (A detailed paragraph about the study's purpose, methods, and results). Other sections include Overall design, Contributor(s), Citation missing, NIH grant(s), Submission date (Feb 06, 2017), Last update date (Feb 07, 2017), Contact name (Anthony Bertucci), Organization name (University of Bordeaux), and Street address (Place du Dr Peyneau).

RESULTS: Gene expression

Regular approach : Downstream vs Upstream

Only 41 Differentially Expressed Genes !
Many / Confounding factors + Large variability

RESULTS: Gene expression



Regular approach : Downstream vs Upstream

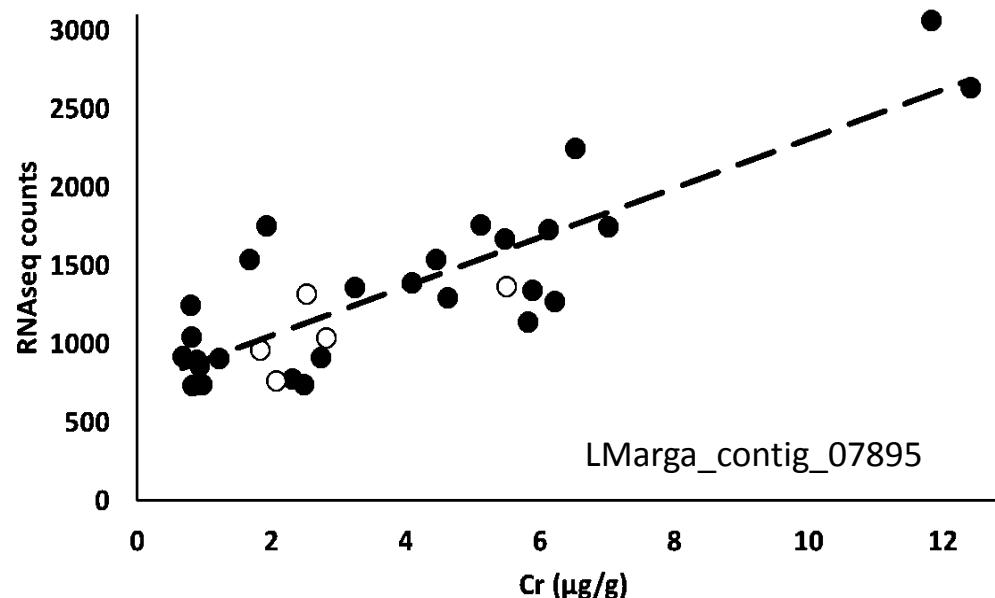
Only 41 Differentially Expressed Genes !
Many / Confounding factors + Large variability

Alternative approach : Factorial Analysis for Multiple Testing (Causeur *et al.* 2011)

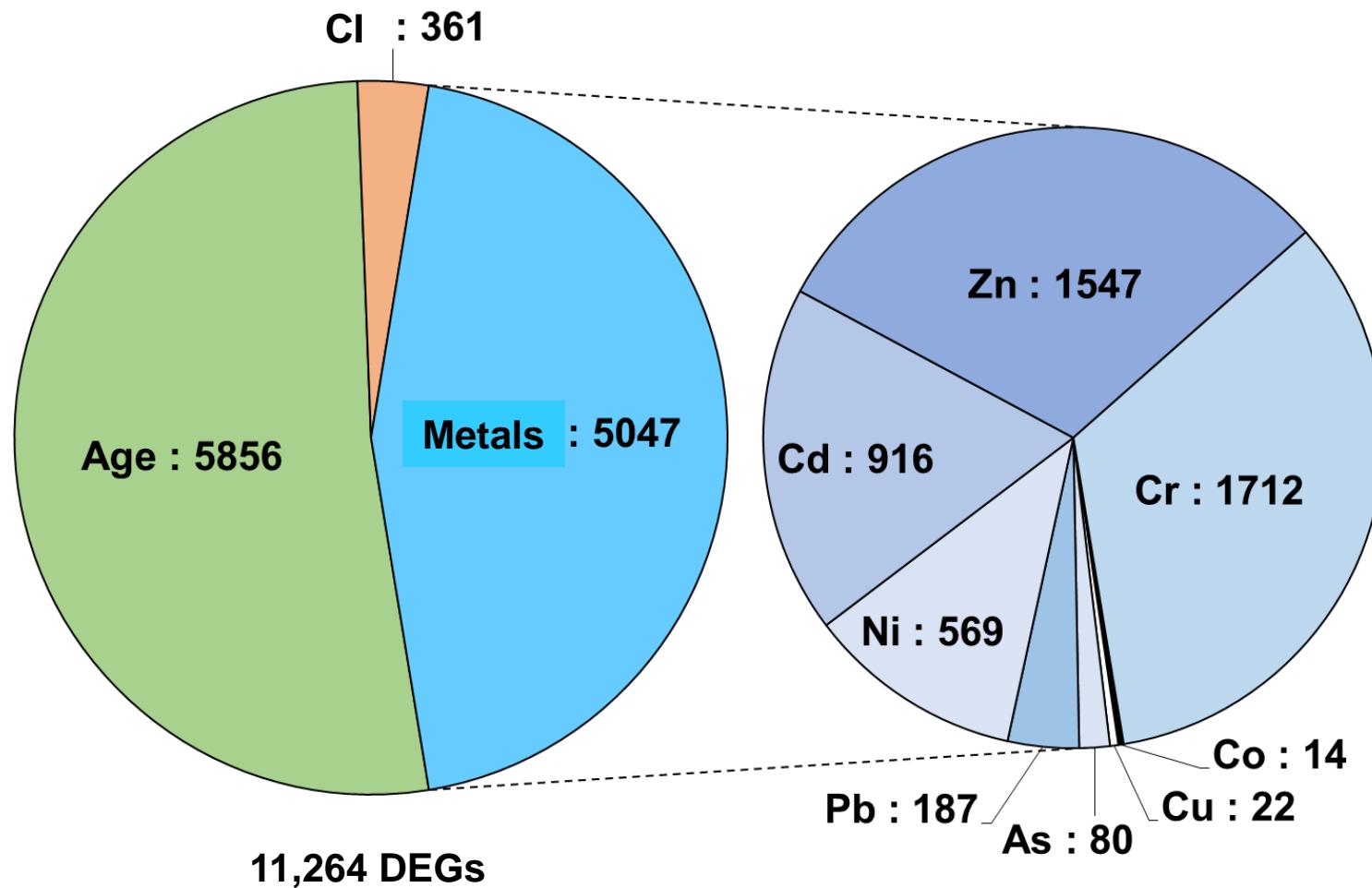
Covariates matrix → 32 individuals x 11 variables

Expression matrix → 32 individuals x 51,392 transcripts

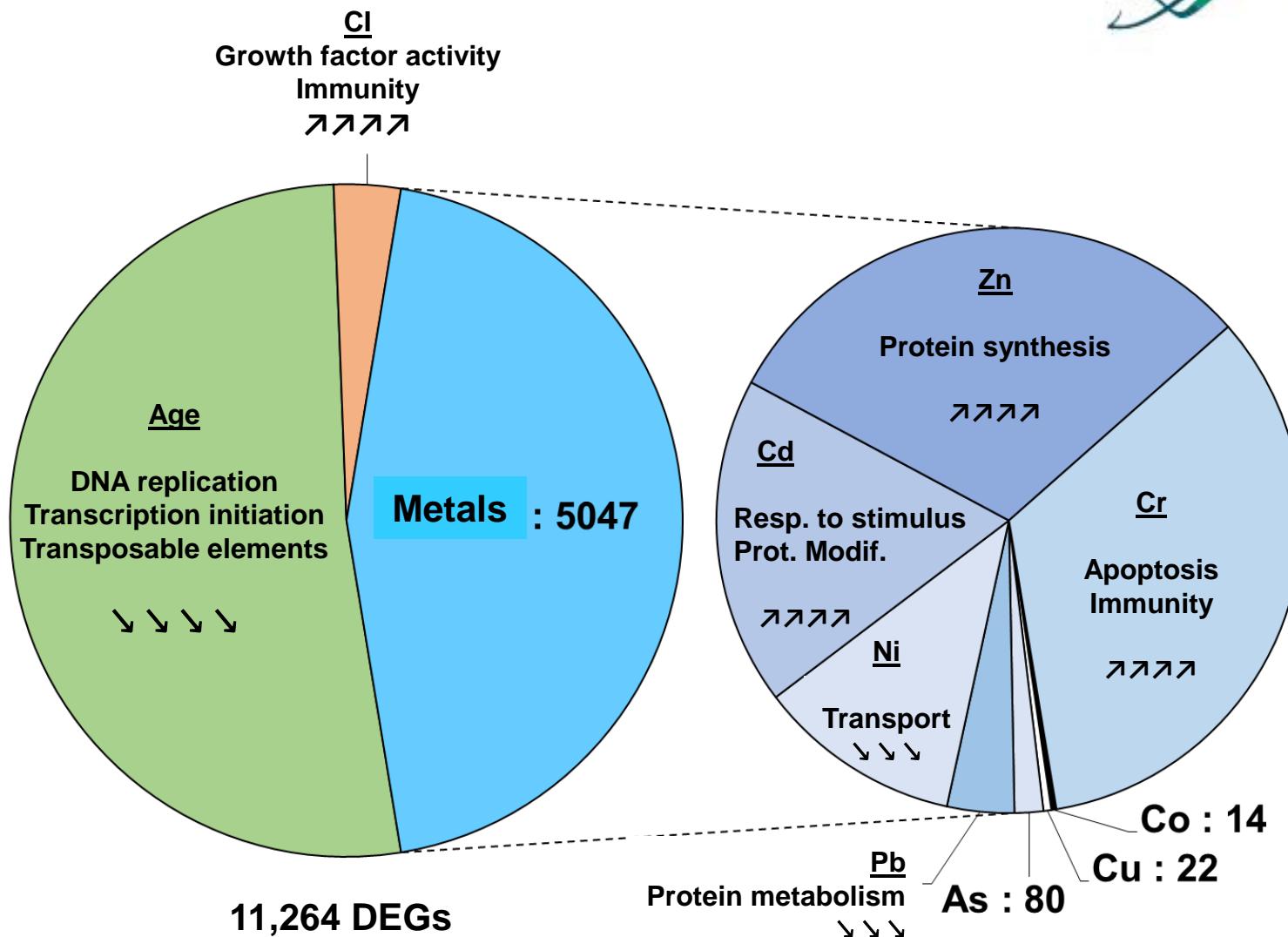
Purpose : Find correlations between gene expression and covariates
What factor affects gene expression ?



RESULTS: Gene expression



RESULTS: Gene expression



RESULTS: Gene expression and Age

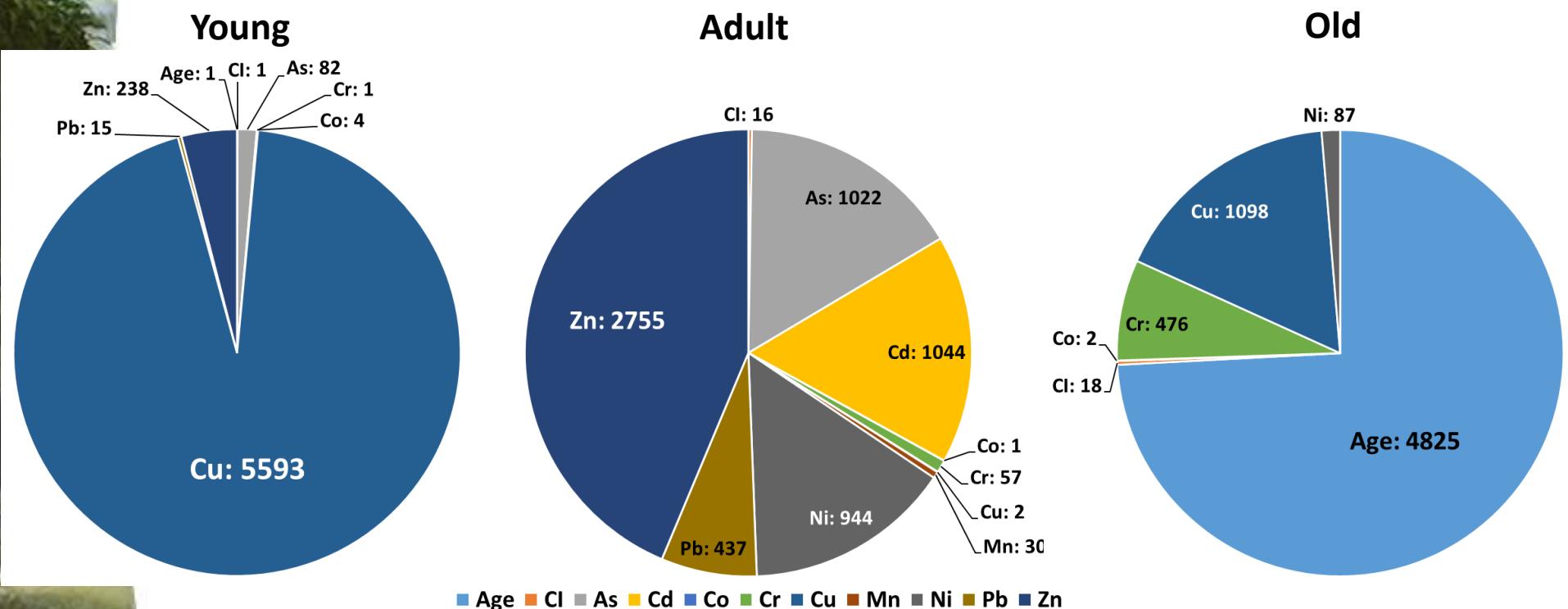


Category	n	Min. age	Mean age	Max. age
Young	10	7	12	21
Adult	11	25	34	43
Old	11	47	53	67

RESULTS: Gene expression and Age



Category	n	Min. age	Mean age	Max. age
Young	10	7	12	21
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Laboratory experiment

Control
Upstream
landfill

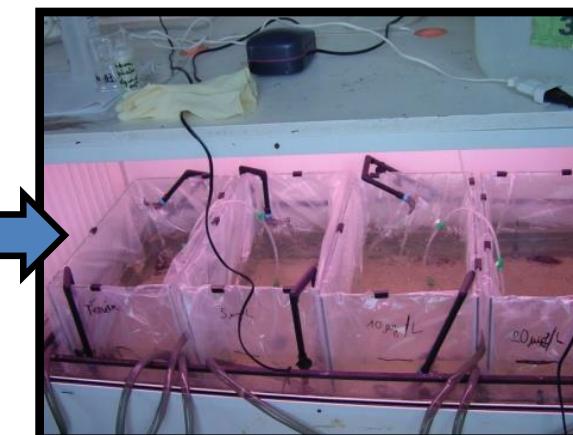
Control
Downstream
landfill

Cadmium
 $2 \mu\text{g/L}$

Cadmium
 $5 \mu\text{g/L}$

Estradiol

7 cm sand
Dronne water
6 individuals per unit
 $T^\circ\text{C} = 18.76 \pm 0.13^\circ\text{C}$
 $\text{O}_2 = 9.11 \pm 0.03 \text{ mg/L}$
 $\text{pH} = 7.20 \pm 0.05$
16h light / 8h dark

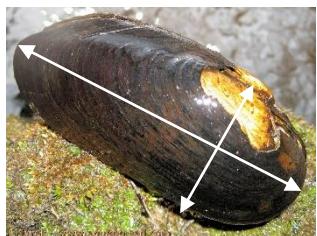


14 days acclimation
7 days exposure

→ 30 individuals in experimentation in july 2009

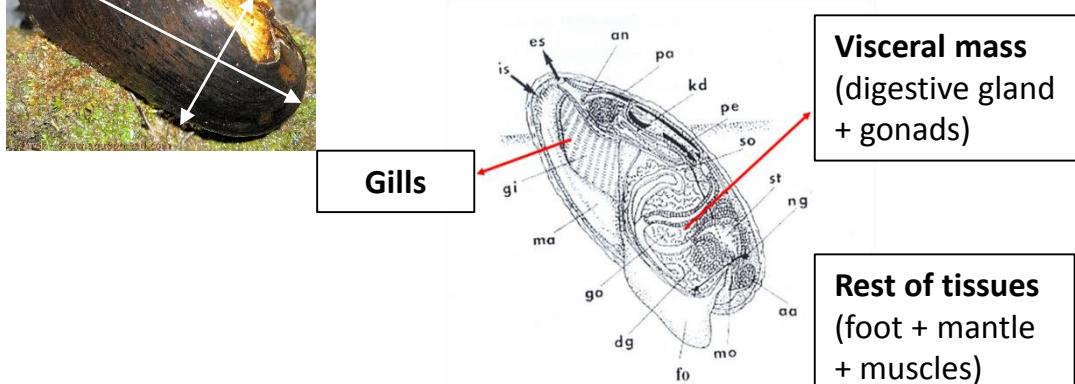
Analyzed parameters

- Morphometric measures



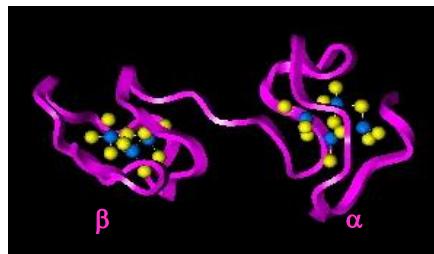
Gills

- Microscopy of the gonads



- Bioaccumulation of metals in organs

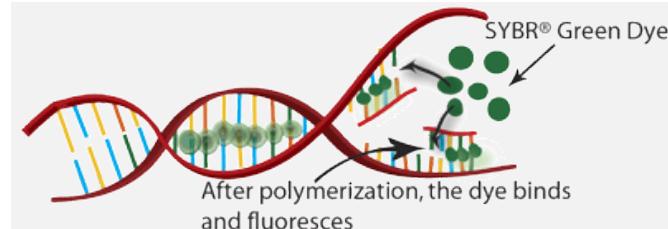
- Metallothioneins quantification



Metallothionein of mammals
 ● Cystein
 ● Cadmium

- Malondialdehyde dosage

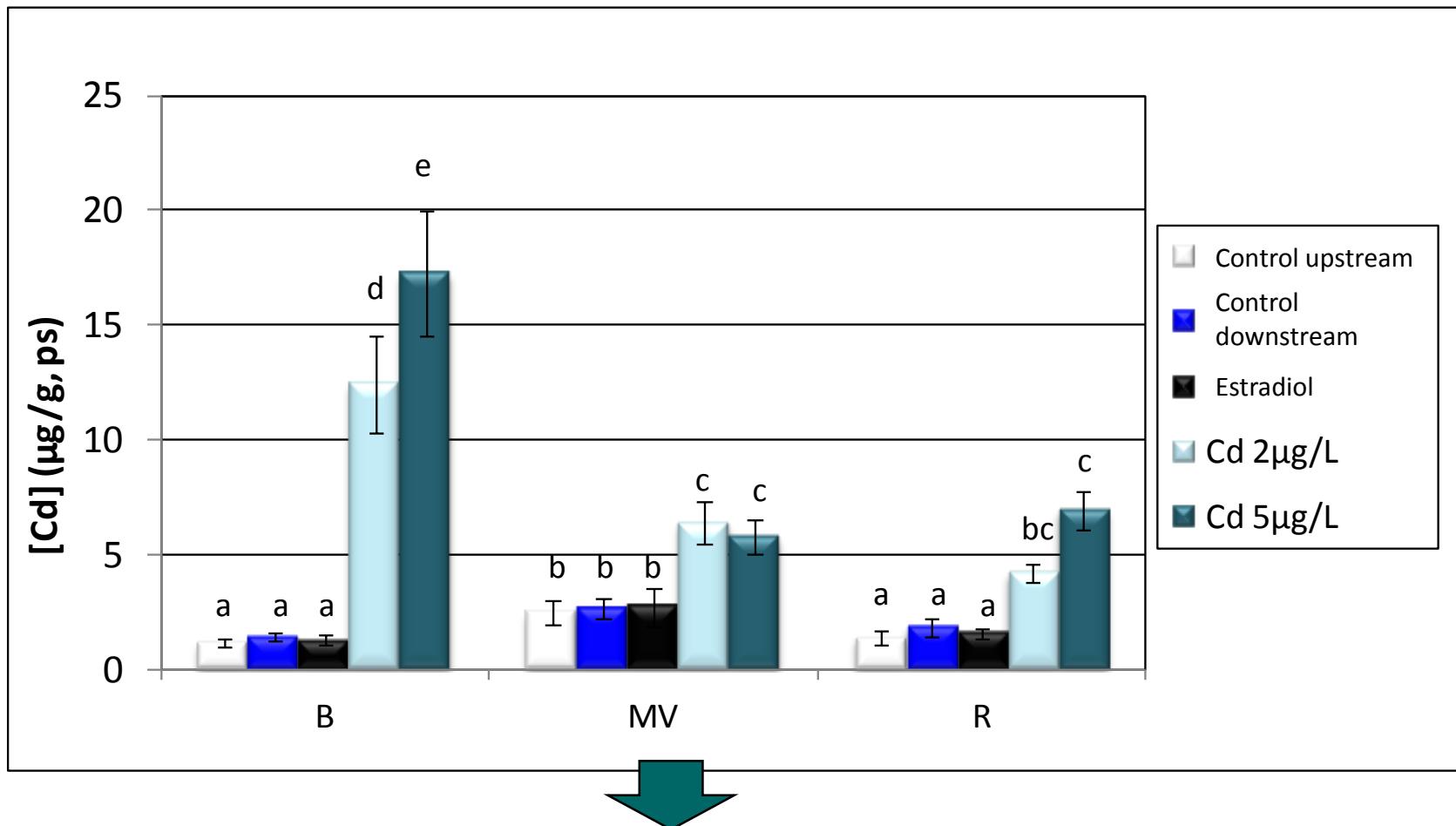
- Quantitative relative gene expressions (ARNm)



Results and discussion



Bioaccumulation of cadmium (Cd)

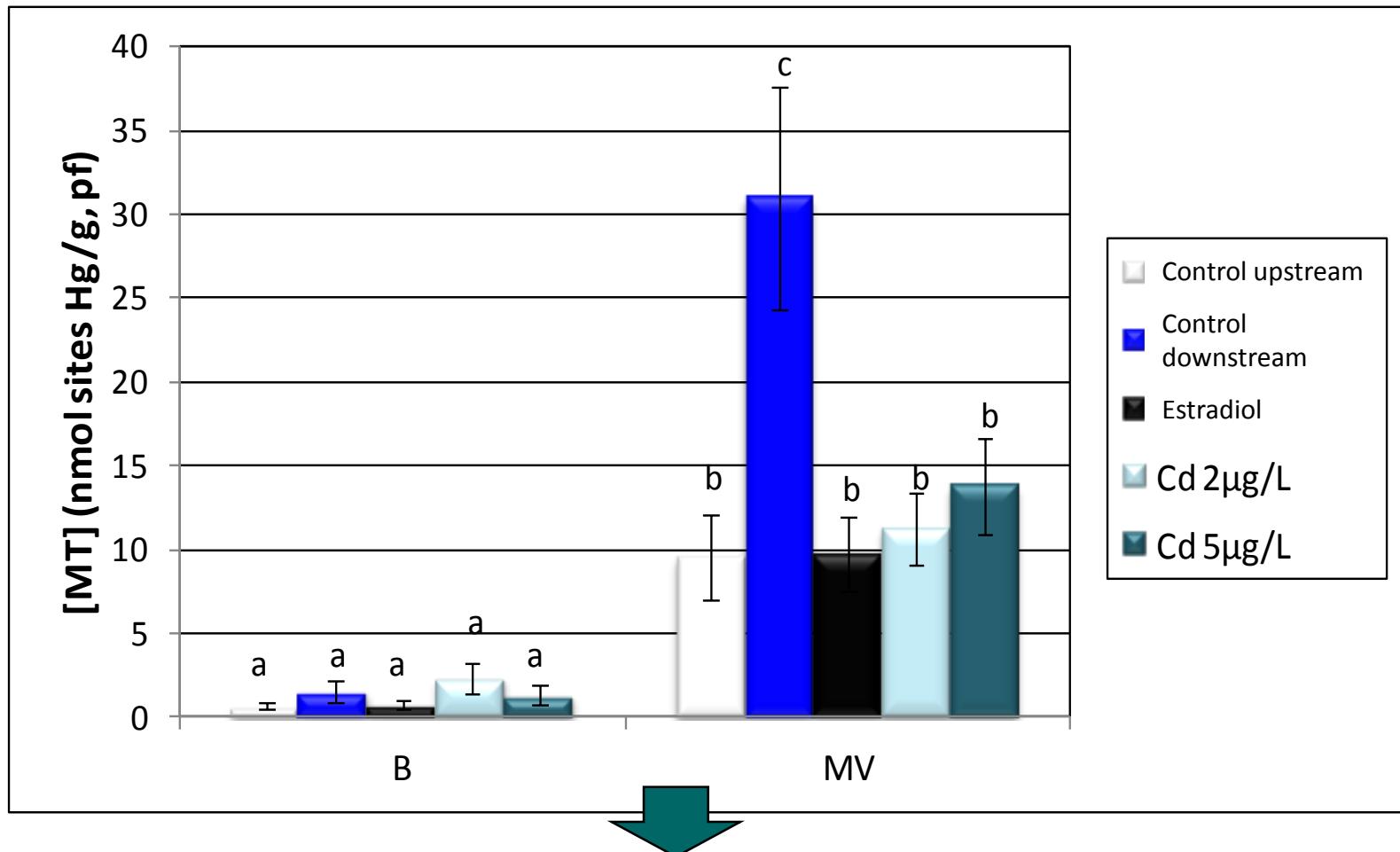


Significant accumulation of Cd in conditions from $2 \mu\text{g/L}$ with a weak concentration effect

Results and discussion



Metallothionein concentrations (MTs)



No response of MTs with Cd in laboratory, but significant response of MTs in the visceral mass in condition Control downstream

Results and discussion



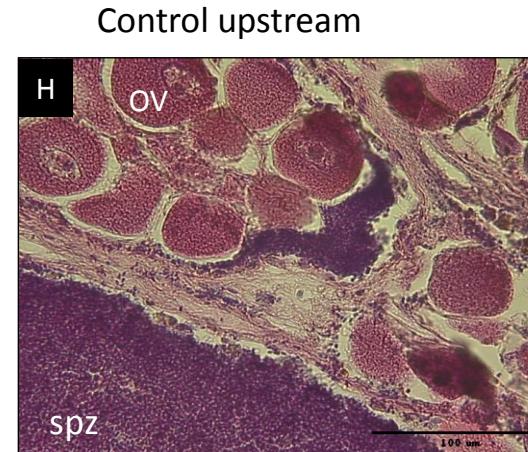
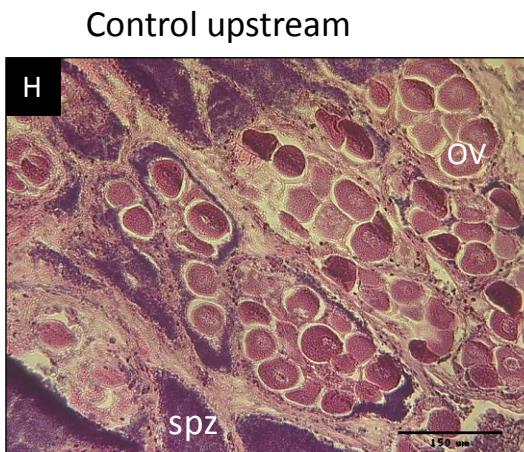
Quantitative gene expression

Function	Gills	Downstream/ Upstream	Est/Upstream	2µgCd/Upstream	5µgCd/Upstream
Lutte contre le stress oxydant	<i>sod</i>	0,51	0,15	0,34	0,12
	<i>sodMn</i>	6,00	1,34	0,62	0,55
Métabolisme mitochondrial	12S	1,76	3,85	0,56	7,63
	<i>coxl</i>	3,31	1,36	4,41	19,51
Détoxication	<i>mt</i>	36,32	0,99	3,65	18,12
Cd effect	Visceral mass	Downstream/ Upstream	Est/Upstream	2µgCd/Upstream	5µgCd/upstream
	<i>sod</i>	0,47	0,72	0,71	0,64
	<i>sodMn</i>	1,94	4,33	6,05	1,77
	12S	16,88	2,26	0,96	0,64
	<i>coxl</i>	10,51	6,52	3,41	7,44
	<i>mt</i>	15,19	1,60	4,77	9,38
	Kidney	Downstream/ Upstream	Est/Upstream	2µgCd/Upstream	5µgCd/upstream
	<i>sod</i>	0,51	0,04	0,81	0,48
	<i>sodMn</i>	1,08	0,80	0,33	0,73
	12S	13,67	0,17	0,82	1,37
	<i>coxl</i>	3,31	2,61	35,25	181,20
	<i>mt</i>	1085,04	0,57	6,81	22,48

Histological analyses of gonads

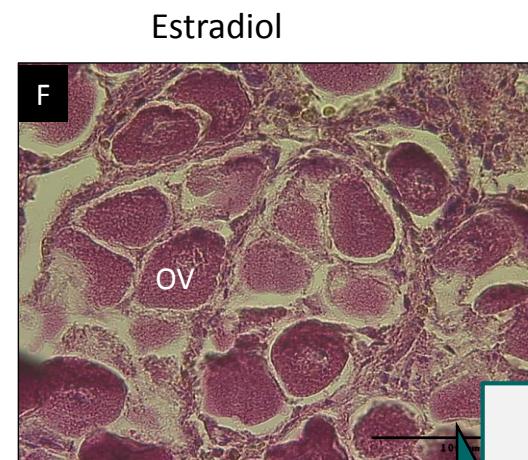
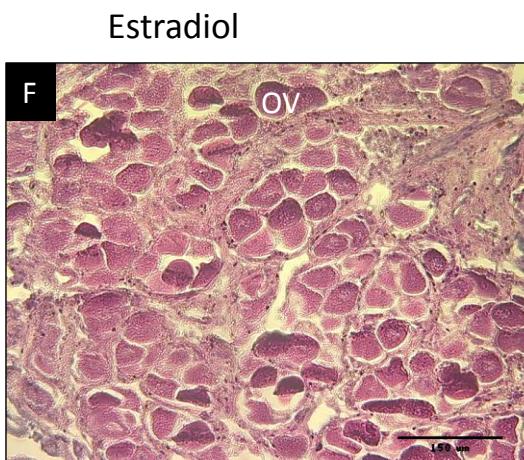


Hermaphrodite



0% of female

Female



33% of female

Female



**Cd mimics
Estradiol effect:
Endocrine
disruption**

33% of female

Conclusion & Perspectives



Classical gene expression approach:

Small number of differentially expressed genes

In situ studies:

Multiple confounding factors

Factorial Analysis:

Correlation gene expression / covariates
Specific markers
Importance of age

Endocrine disruption of Cd

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What next ?

Rearing facility: >90,000 recruits / year

Controlled conditions

Mussels of the same age



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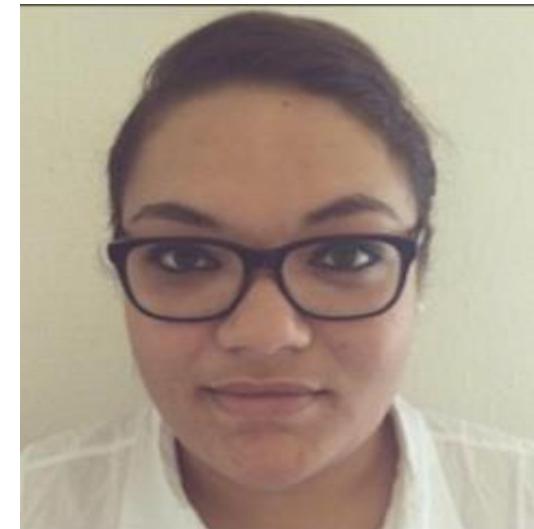
Endocrine disruption of Cd

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PhD of Tiare Belamy

Thank you for your attention!



<http://ngspipelines2.toulouse.inra.fr:9007/>

NCBI GEO: GSE94542





Le programme LIFE 13 NAT / FR / 000506

Préservation de *Margaritifera margaritifera* et restauration de la continuité écologique de la Haute Dronne

Co-portage : PNR Périgord-Limousin / UMR EPOC
(Université Bordeaux / CNRS)

Durée de 6 ans (Juin 2014 – Mai 2020)

Budget : 5 855 204 €

Site web : <http://www.life-haute-dronne.eu/>



Résultats et discussion



Expression quantitative des gènes

Fonction	Branchies	Aval/Amont	Oest/Amont	2µgCd/Amont	5µgCd/Amont
Lutte contre le stress oxydant	<i>sod</i>	0,51	0,15	0,34	0,12
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Aval par rapport à l'amont

- Stress oxydant
- Perturbation du métabolisme mitochondrial
- Détoxication

	Masse viscérale	Aval/Amont	Oest/Amont	2µgCd/Amont	5µgCd/Amont
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Fonction	Rein	Aval/Amont	Oest/Amont	2µgCd/Amont	5µgCd/Amont
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Effet de l'oestradiol

- Stress oxydant
- Perturbation du métabolisme mitochondrial



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