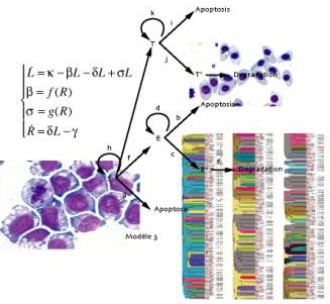

SEMOVI

September 7, 2010

Aquatic ecosystems :

tools for contaminant exposure assessment and effect diagnosis in ecotoxicological survey

Example of a genotoxicity biomarker

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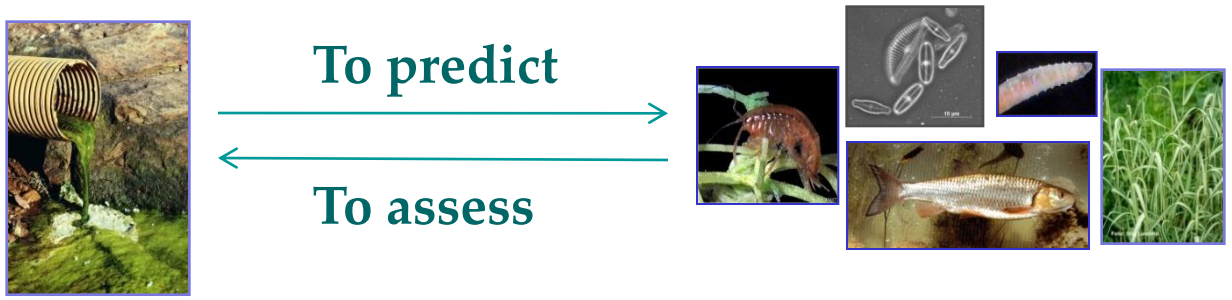
Cemagref Sciences, eaux & territoires

INRA Institut National de la Recherche Agronomique

How to assess the ecotoxicological risk ?

Biological parameters have been proposed to complement the information given by chemical analyses



Biomarkers, bioassays, bioindicators

European Water Framework Directive :

Assessing the impacts of pressures on water bodies in terms of **ecological and chemical impacts**

Early warning indicators

respond **before measurable effects** on individual performance and population/community

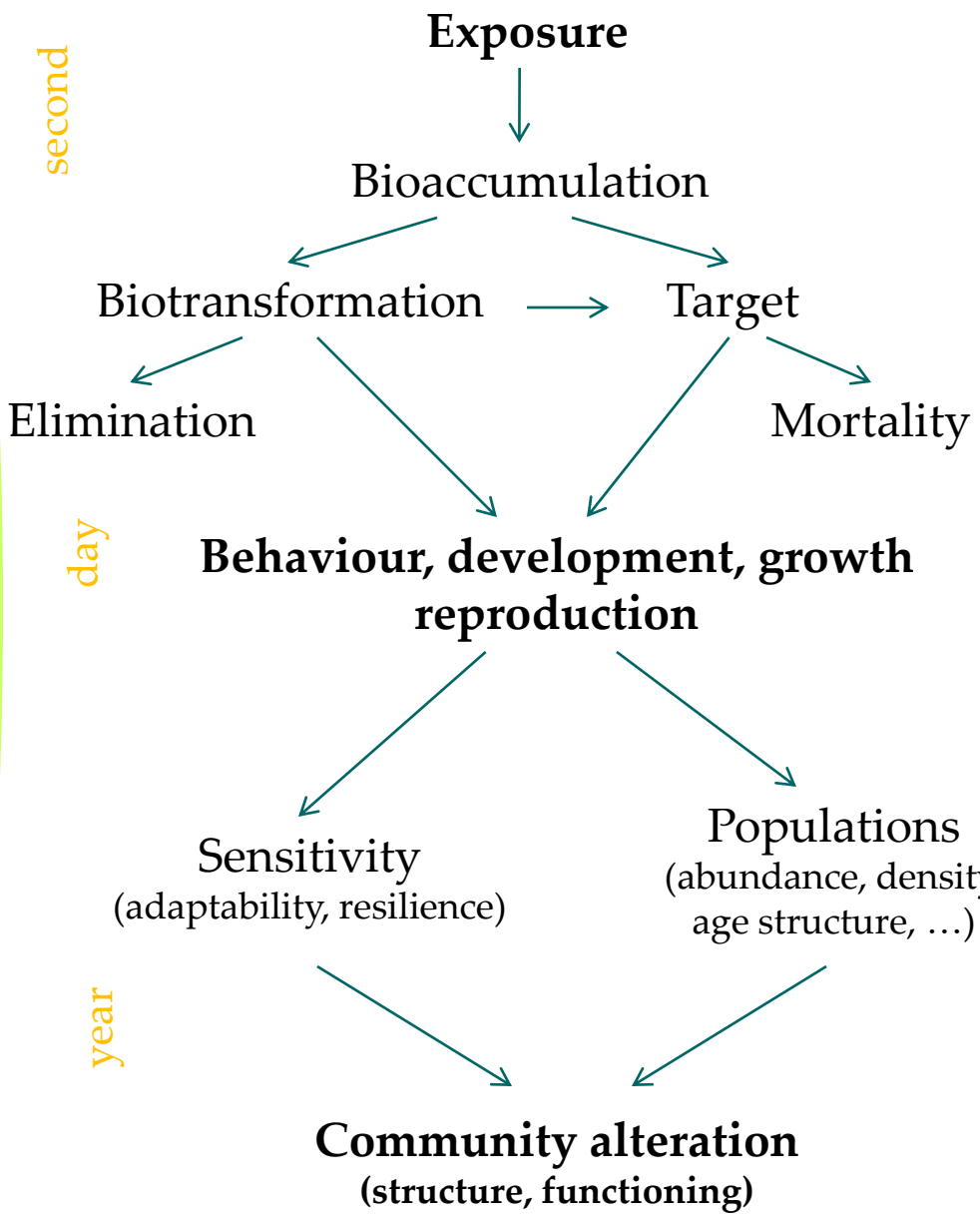
Their aims :

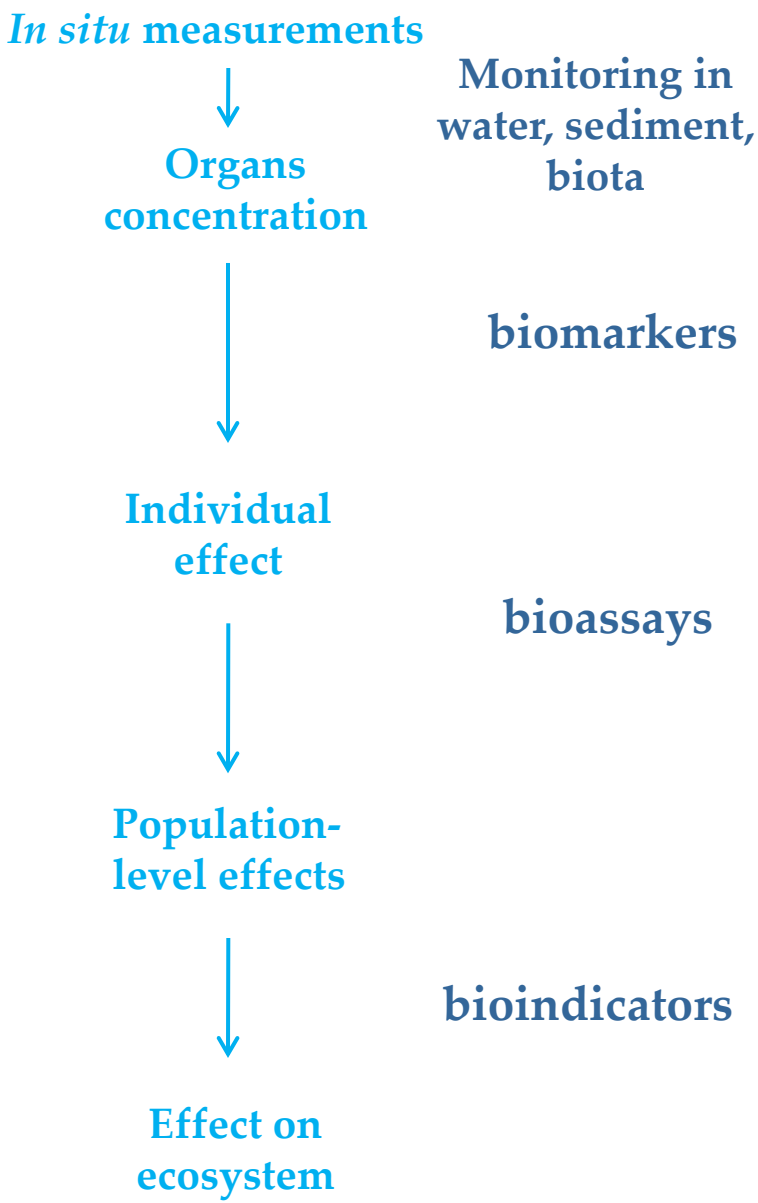
- to indicate that **organisms have been** or are being **exposed** to certain chemicals,

- to indicate that organisms are suffering and/or likely will suffer leading to future **impairments of ecological relevance,**

- to aid to **identify the causes** of population and community level effects that are observed.

From Lagadic and Caquet, 1996

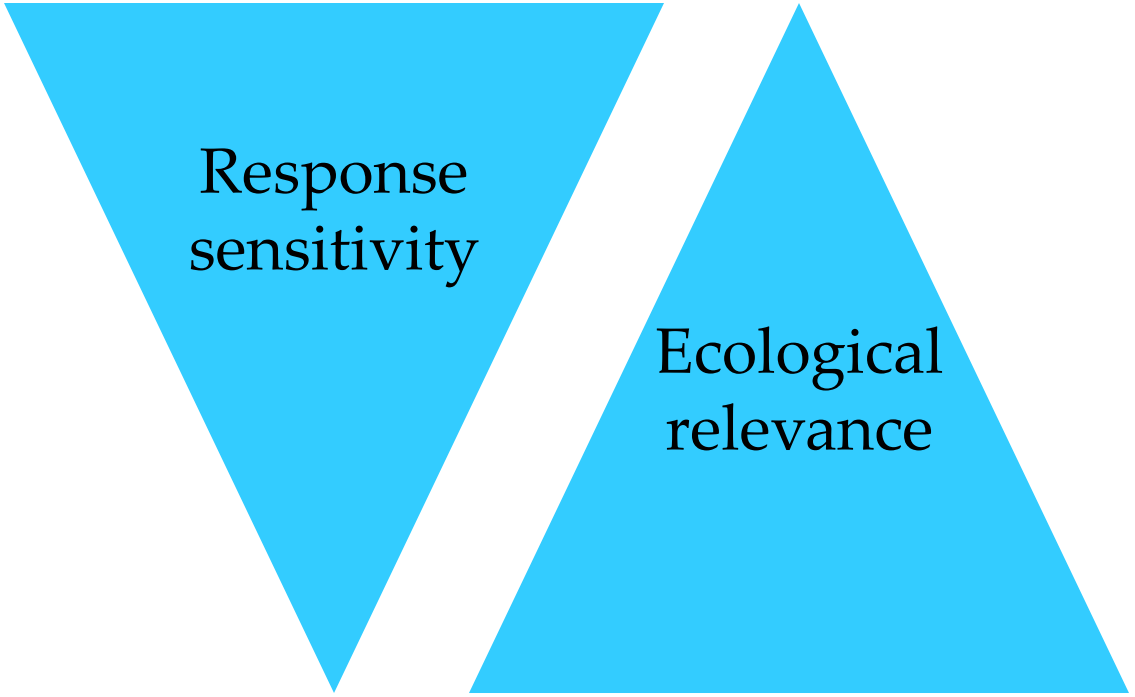


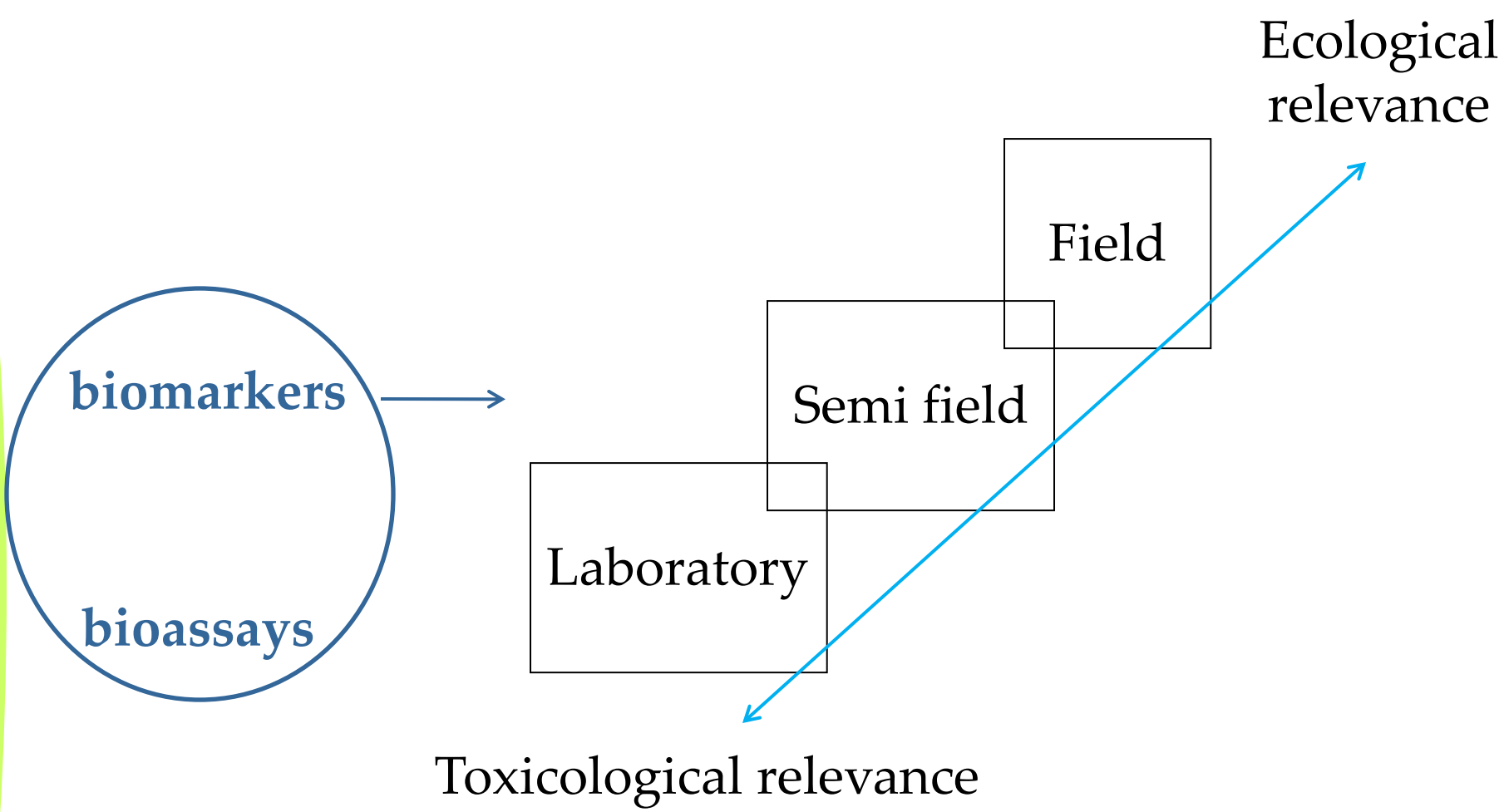


biomarkers

bioassays

Bioindicators





Bioindicators

From Caquet, 1997

Precocity
Specificity
Replicability

LABORATORY CONDITIONS

isolated species

simplified food-web

microcosm

mesocosm

artificial stream

experimental ponds

enclosure

OUTDOOR CONDITIONS

natural ecosystems

From Caquet, 1997

Precocity
Specificity
Replicability

Ecological relevance
Complexity
Realism

LABORATORY CONDITIONS

isolated species

simplified food-web

microcosm

OUTDOOR CONDITIONS

mesocosm

artificial stream

experimental ponds

enclosure

natural ecosystems

From Caquet, 1997

Models
Decision-aiding tools

Development

Improvement

Proof

Precocity
Specificity
Replicability

Ecological relevance
Complexity
Realism

LABORATORY CONDITIONS

OUTDOOR CONDITIONS

isolated species

simplified food-web

microcosm

mesocosm

artificial stream

experimental ponds

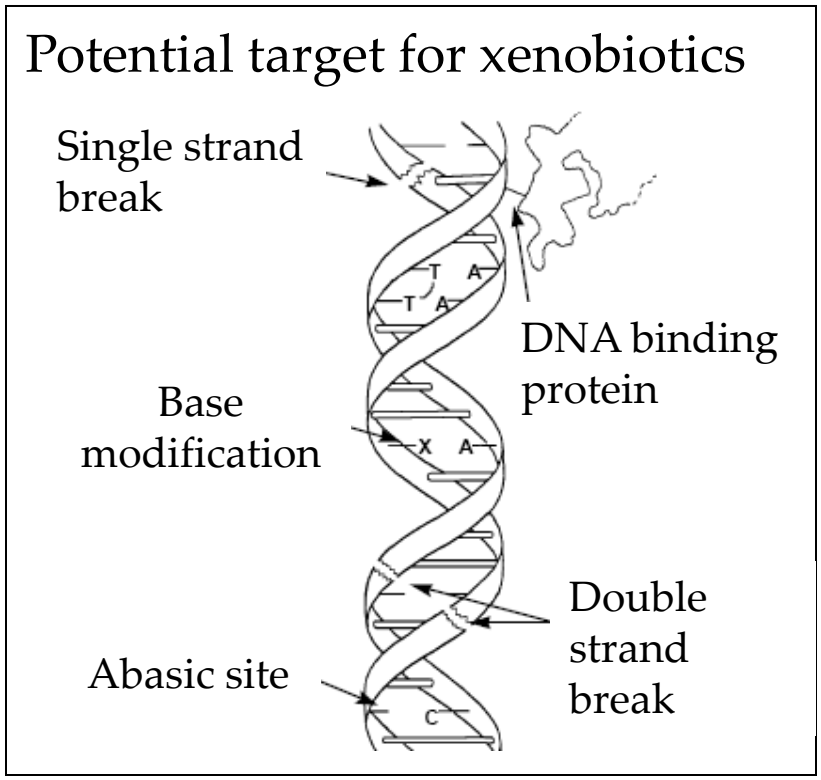
enclosure

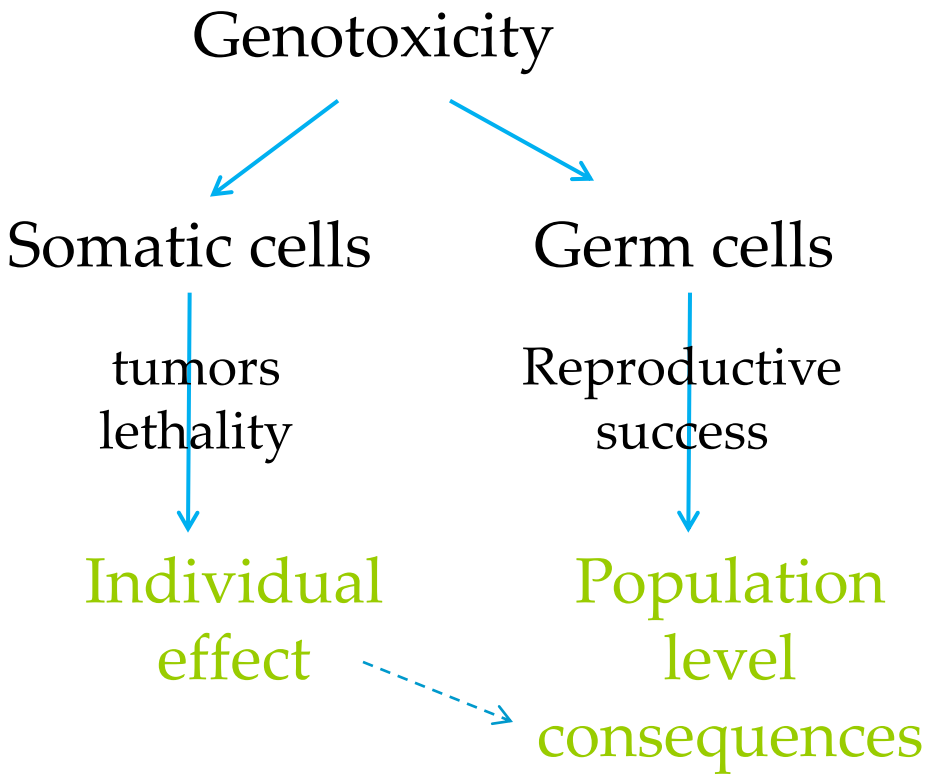
natural ecosystems

Development, validation and significance of a genotoxicity biomarker

Why a genotoxicity biomarker ?

DNA: physical carrier of inheritance





How do we have proceed ?

A tool to provide unambiguous and ecologically relevant response of exposure to or effects of toxicants…

3 steps

- a **standard** procedure
- **confounding influences** : biotic and abiotic factors
- establish a **link with a higher level** of biological effects (fitness)

Gammarus fossarum

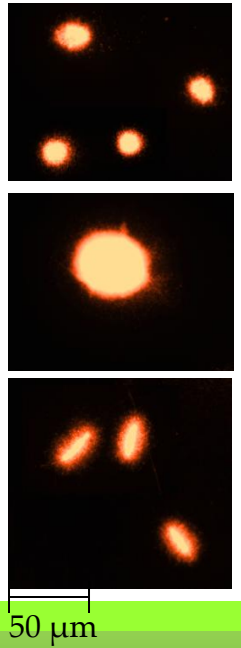
widespread and abundant in Europe

known to be sensitive to a large range of stresses

currently used in ecotoxicological tests

plays a **major role in the entire food web**

DNA damage: primary damages assessed by the Comet assay



Haemocytes → role in the transport of toxicants and in various defence mechanisms

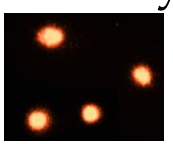
Oocytes

Spermatozoa

→ propagate the DNA used for the development of the next generation

Choice of a relevant cell type to assess DNA damage :

In vitro, in vivo and *in situ* experiments

	Haemocytes 	Oocytes 	Spermatozoa 
Low DNA damage in the control	+ +	+	+ +
Low variability between replicates	+	-	+
Significant dose-dependent relationship	+	+	+
Lack of repair capacity	-	-	+
Ability to distinguish genotoxic impact in the field (WWTP effluent)	-	-	+

Spermatozoa

Exhibit the **highest genotoxic response**,

partially because of **lower repair capacity**,

making them the most **integrative and sensitive cell type**

Relevant cell type for genotoxicity assessment in the field

How to avoid the misinterpretation of our biomarker of genotoxic impact ?

DNA damage in *G.fossarum* sperm: **response variability and reference level**

I. Response variability

impact of **biotic** factors: impact of sperm maturation on DNA damage

laboratory

knowledge of response kinetics

laboratory/field

II. Reference level

impact of the main **abiotic** factors : temperature and conductivity

laboratory

spatio-temporal variability of basal level in wild populations

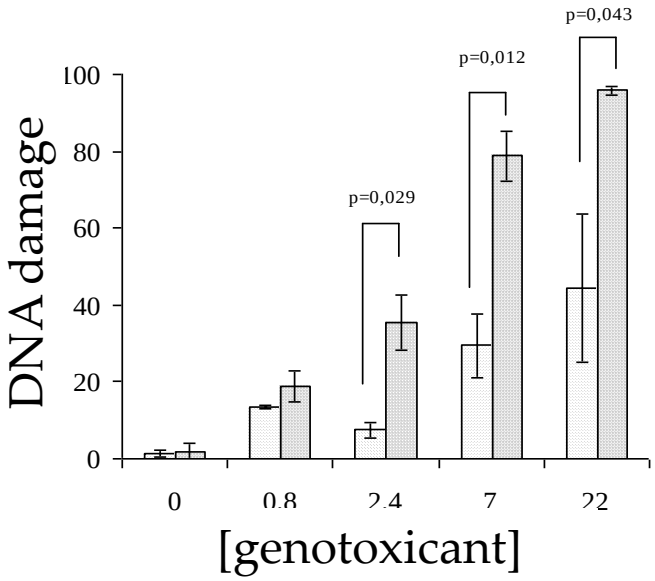
field

I. Response variability

-Choice of physiological status of test organism: impact of sperm maturation

Early maturation

End of maturation



→ Test organism = male at the last stage of spermatogenesis

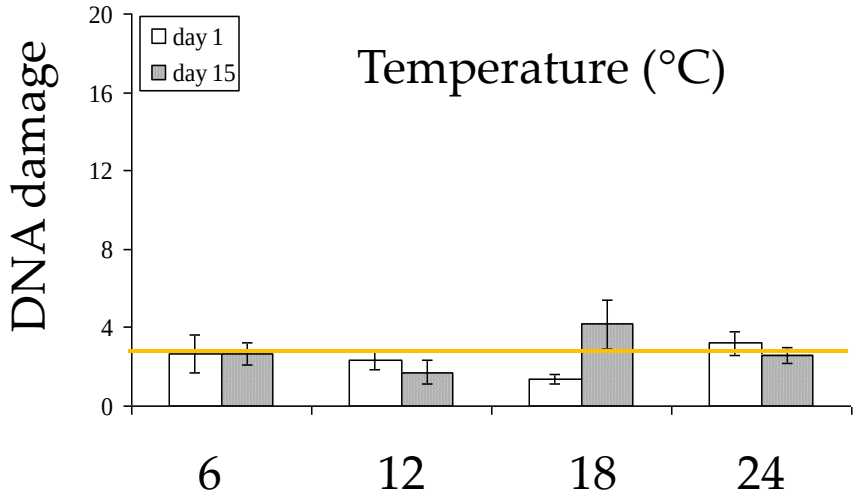
-Knowledge of response kinetics

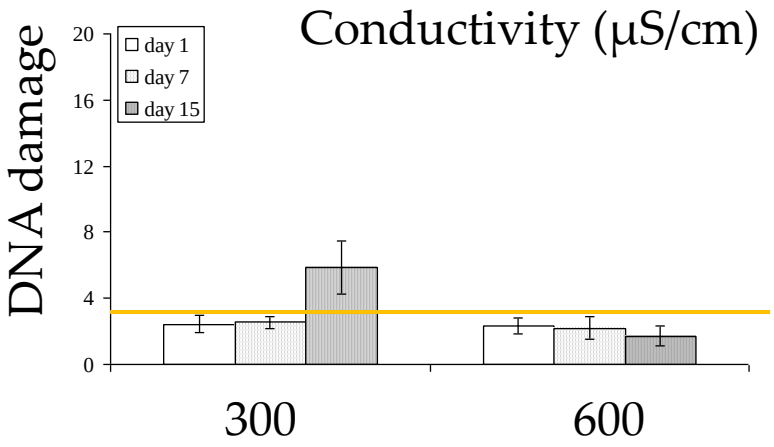
DNA damage linked to exposure duration but not in a linear way

7 day exposure is a relevant time duration (response plateau, spermatogenesis)

II. Reference level

Impact of the main **abiotic factors : temperature and conductivity**



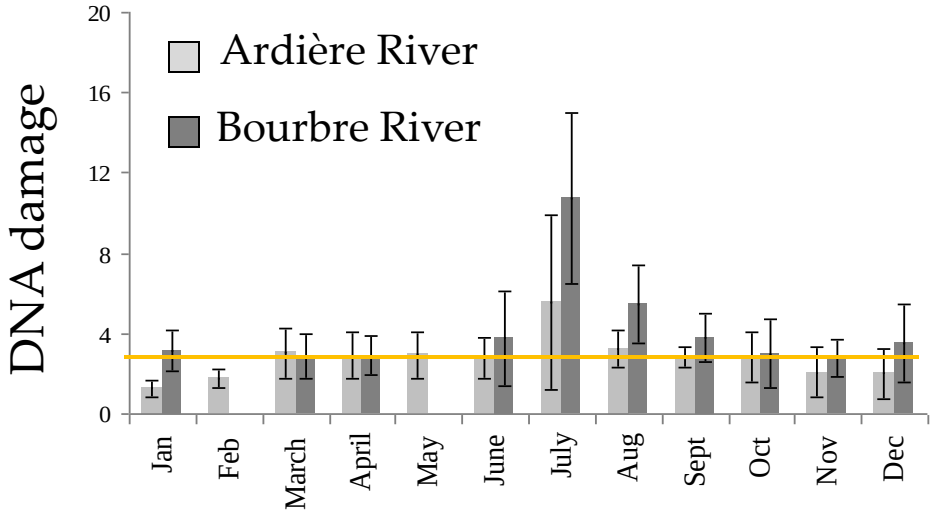


No impact of temperature

No impact of conductivity

II. Reference level

Spatio-temporal variability of basal level in 2 wild populations

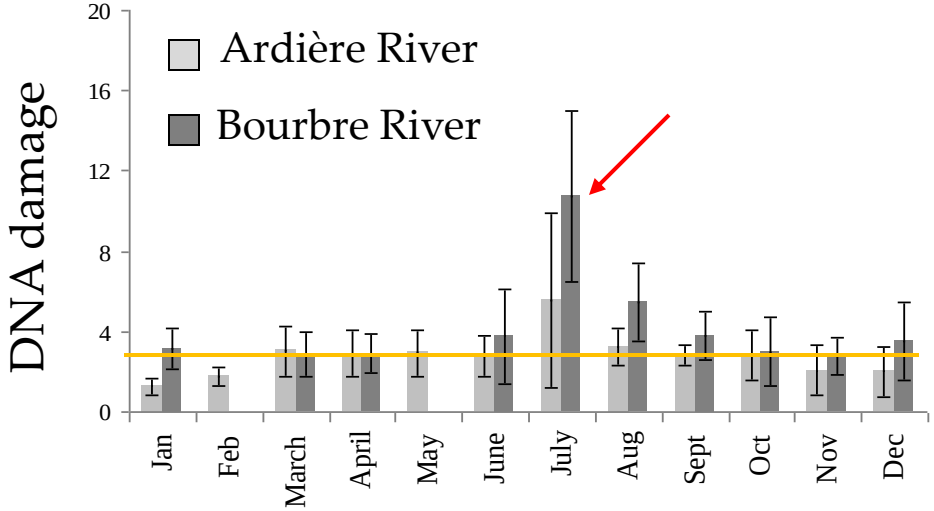


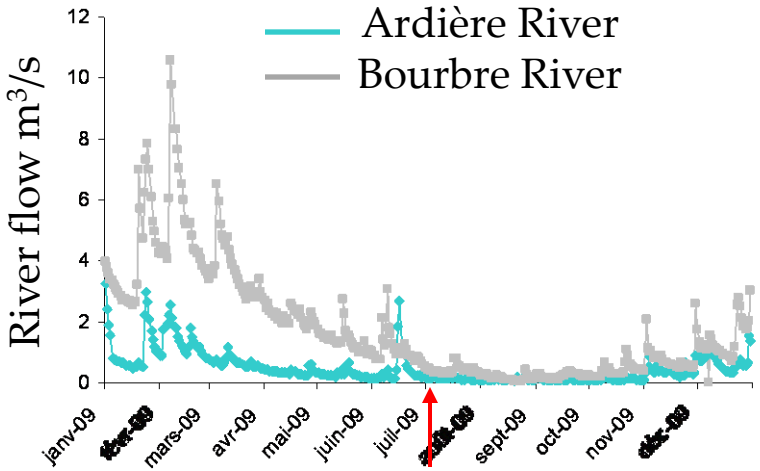
No impact of the **watersheds**

No clear **seasonal variability**
(except during the warmest months : T°C around the lethal value)

II. Reference level

Spatio-temporal variability of basal level in 2 wild populations





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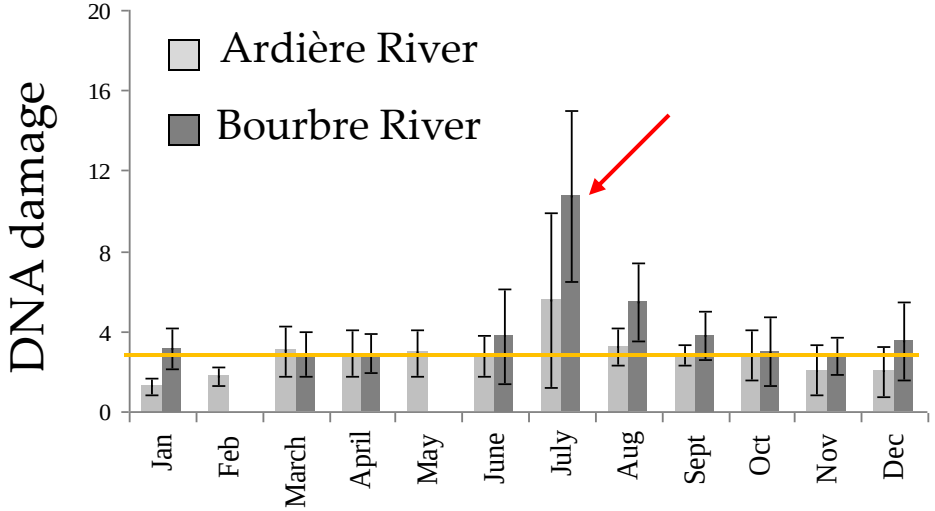
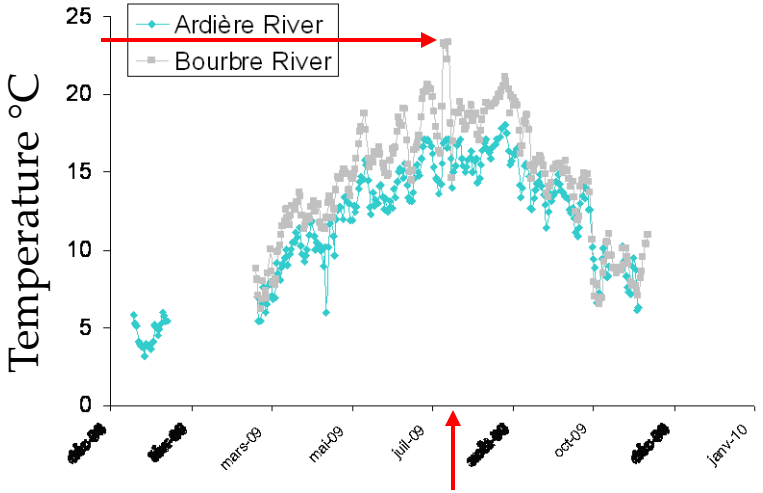




No impact of the **watersheds**

No clear **seasonal variability**
(except during the warmest months : T°C around the lethal value)

Have we chosen the most relevant or enough abiotic factors (O_2) ?

Could statistical models validate these factors ?

DNA damage in *G.fossarum* sperm: **intrinsic variability and reference level**

I. Response variability

Intrinsic variability
-reproductive status of *G. fossarum*
-DNA damage
= f(exposure duration)

II. Reference level

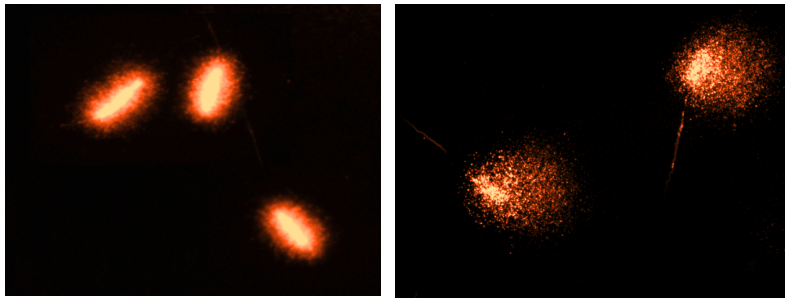
Reference level : 3% DNA damage
Seasonality and watershed have negligible impacts

Can we assess genotoxicity in the field ?

Preliminary **reference control value** beyond which the damage may be attributed to **contaminant exposure in the field**

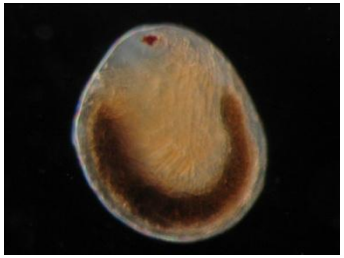
Could we link genotoxic responses in *G. fossarum* germ cells with reproduction impairment ?

From sub-individual to individual responses



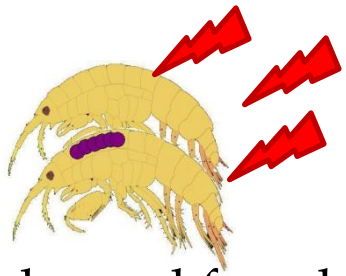
Sperm DNA integrity
relevant exposure biomarker

Cascade of adverse changes from the cellular to the organism levels



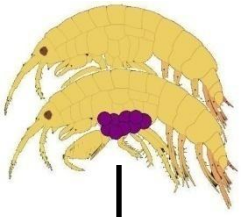
Empirical link between sperm DNA damage and mbryotoxicity ?

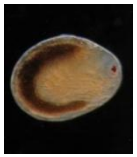
2 genotoxicants having different mode of action



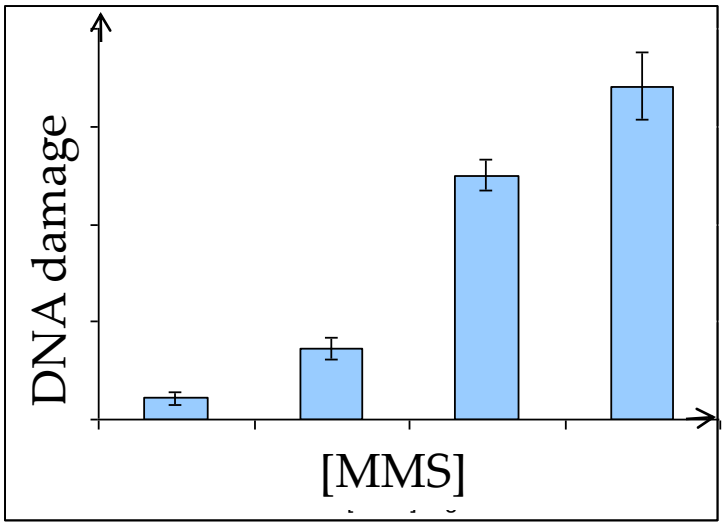
Males and females exposure before spawning

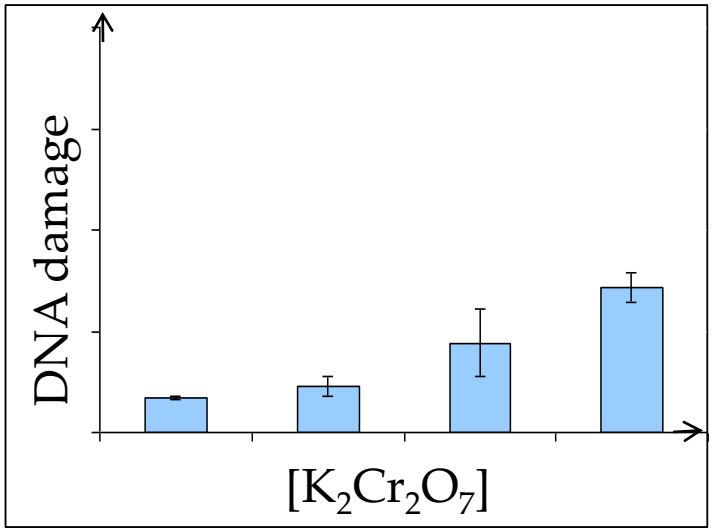
DNA damage in germ cells



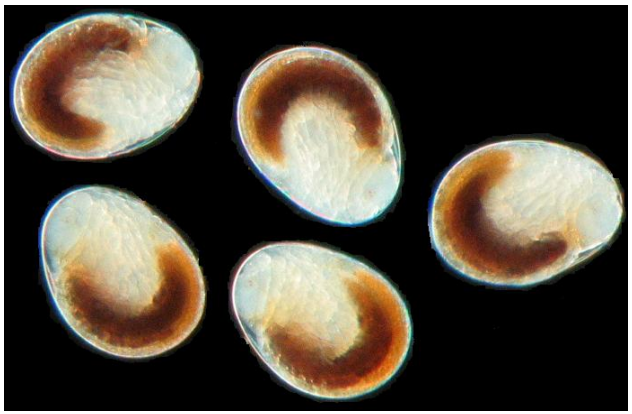


embryos development in non contaminated water

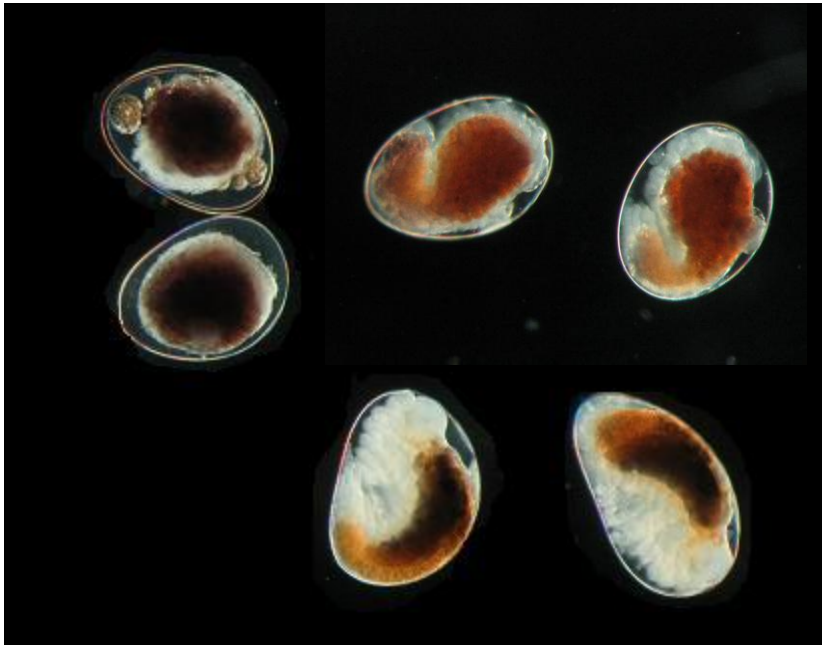




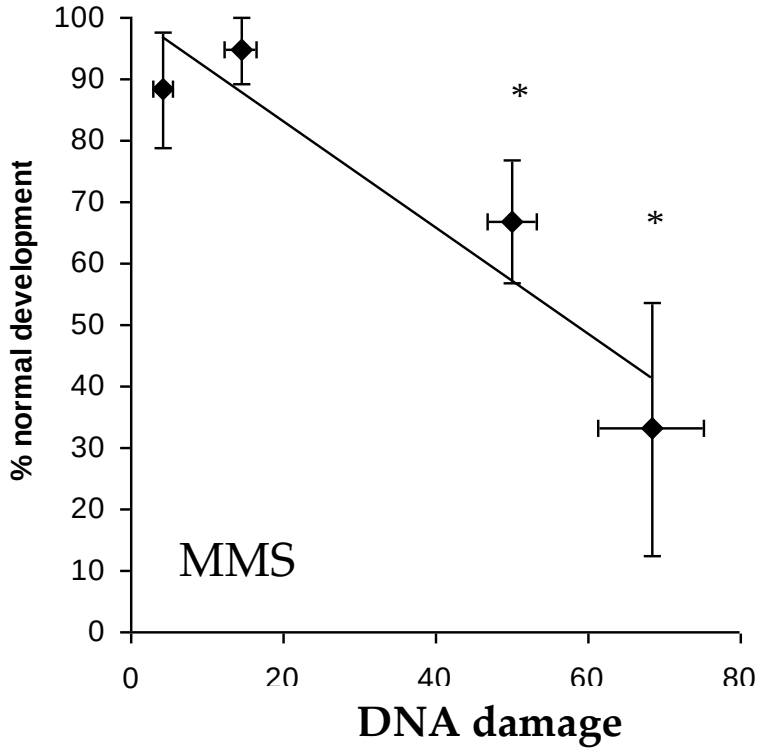
DNA damage in spermatozoa

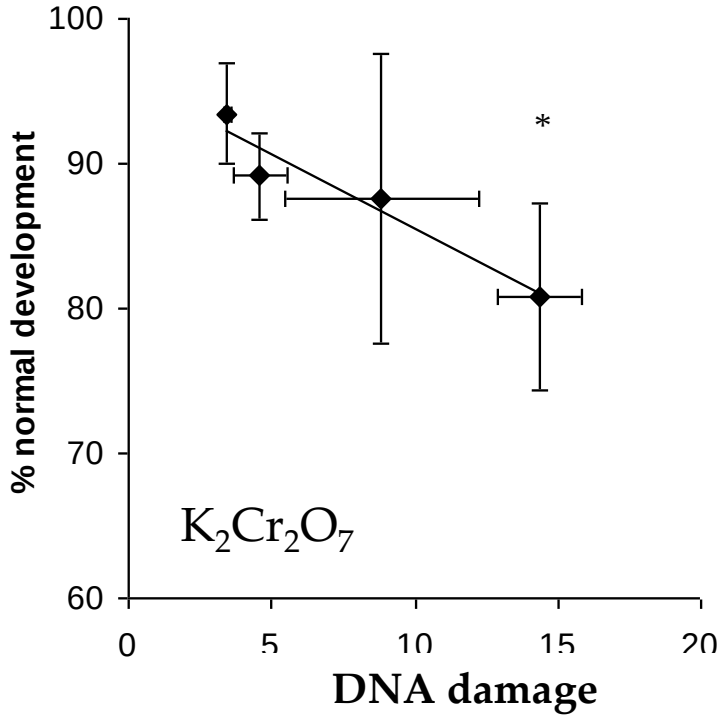


Normal embryos
After 21 days of development at 12°C



Abnormal embryos
After 21 days of development at 12°C





Significant DNA damage in spermatozoa was correlated with increasing embryo abnormalities

→ **Statistical link between molecular and individual response**

Can such statistical correlations still exist in other context ?

→ **Integrated mechanistic models could be developed ?**

Conclusion

- Validation of a new biomarker for this crustacean
- Establishment of a **basal level value** (confounding factors)

- **Spatial scales:** significant discrimination of potentially genotoxic sites with caging experiments
- **Biological scales:** relationship bridging the biomarker response and its consequences at the individual level

BUT **which ecological relevance** could be given to DNA damage since the only one measurement of sperm DNA integrity cannot predict the reproductive success **in the field** ?

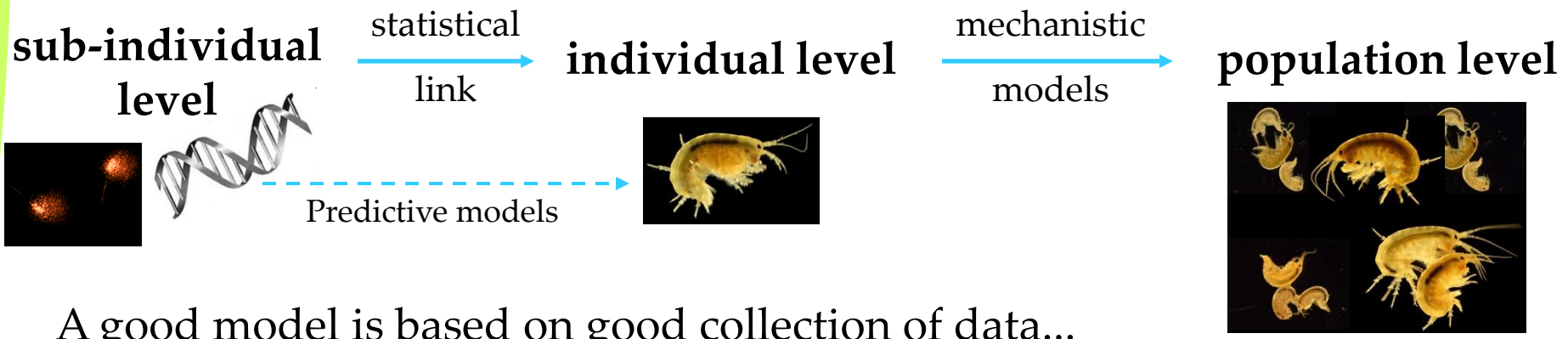
Conclusion

Similar approach could be use to develop other tools for environmental risk assessment, nevertheless attention has to be paid to several bottlenecks.

> « Biomarker responses are not likely to **provide** useful **predictions of effects at higher levels of organization**… » (Forbes et al., 2006)

A solution ?

To develop and test **mechanistic** models that allow predictions of effect at multiple scales



A good model is based on good collection of data...

Thank you

for your attention

Author thanks

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