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Variability of Cd-sensitivity and phylogenetic diversity of field populations throughout the *Gammarus fossarum/pulex* species complex.



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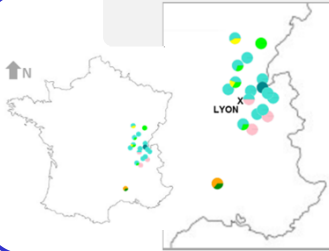
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Context: Gammarids from the pulex group are commonly used in ecotoxicological tests. This taxonomic group is known to be a highly diversified **complex of morphological and cryptic species**, and affiliation to a lineage is claimed to be a driving factor for toxicant sensitivity. Phylogenetic determinism hence **challenges the reliability of ecological risk assessment** of chemicals since it could contribute to the reported between-population/species variability in response to contamination in gammarids, and jeopardizes the representativeness of toxicity tests performed with one experimental population.

Objectives: Here we aim to evaluate whether there are **sensitivity deviations** to cadmium within the *G. fossarum/pulex* species complex and to assess the potential confounding effect of the **phylogenetic signal** in the sensitivity to this model contaminant in this species group.

1. Studied populations



- Broad sample of 18 field populations
- Populations inhabiting pristine stations
- Populations spread out at a regional scale (in the Rhône-Alpes region, Southeast of France) in various ecological (creeks, mountain brook, streams, floodplains) and geological contexts (limestone, crystalline areas)

Design in order to cover:

- ➔ A large range of physico-chemical characteristics to **take into account confounding factors**
- ➔ The **phylogenetic diversity** of the *G. fossarum/pulex* complex

2. Lineages identification

- Genotyping from the nucleotide sequence of a 710-pb segment of the mitochondrial cytochrome c oxidase subunit I gene (COI)
- At least 12 individuals per population

Fig. 1: COI phylogenetic tree of the *Gammarus fossarum/pulex* complex computed from all individuals analysed; and phylogenetic composition of populations, pie slice proportional to the number of individuals of each lineage.

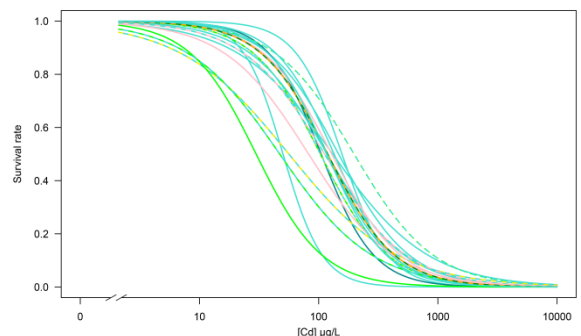


- ➔ 256 individuals; 82 new haplotypes
- ➔ 6 divergent fossarum groups, and 3 pulex groups
- ➔ 9 fossarum B populations, 2 fossarum W populations, 1 pulex population, 6 sympatric cases

3. Cd-sensitivity assessment

- 7 days semi-static exposure to 6 levels of cadmium (Cd) (0, 20, 40, 80, 160 and 320 µg/L, water hardness 140 ± 10 mgCaCO₃/L)
- 3 replicates of 15 males with homogeneous size for each level
- Analysis performed with the drc package in the R software

Fig. 2: Dose-response curve of each population - 4 days of exposure



➔ Differences in sensitivity between populations

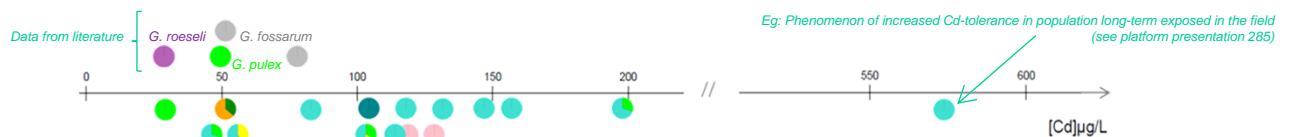
➔ LC50_{96h} of pure-stand populations

	LC50 _{96h} (SD)
<i>G. fossarum</i> B	123,2 (22,4) *
<i>G. fossarum</i> W	124 (6,8) *
<i>G. pulex</i>	29,9 **

* Mean LC50_{96h} of 7 populations for B, and 2 for W
** LC50_{96h} TANC

Conclusions

- ➔ Within *G. fossarum*, Cd-sensitivity of lineages B and W are in the same range. Thus, in the particular case of acute Cd toxicity, we **do not conclude to a phylogenetic signal within *G. fossarum***.
- ➔ We suggest that ***G. pulex* could be more sensitive** than *G. fossarum* but a larger sample of pure-stand populations is necessary in order to conclude about a phylogenetic signal in Cd-sensitivity between these two groups.
- ➔ In addition levels of Cd-sensitivity reported in the literature are in the range of the variability revealed here (Fig 3). It reinforces the importance to study a **broad sample of populations** to ensure that reliable conclusions could be drawn.



- ➔ The variability observed between populations suggests that **environmental factors** may have a leading role in Cd-sensitivity. Local ecological adaptations should be investigated to understand between-population heterogeneity in sensitivity to contaminants in gammarids.

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