Investigation of *Daphnia magna* genotypes with different Zn tolerance using micro-XRF under cryogenic conditions

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In a previous contribution, we illustrated the potential of a cryostream for micro-XRF analysis on *Daphnia magna* close to its native state [1]. *Daphnia magna* is a model organism to investigate the effect of chemical contamination, including metals such as Zn, on ecosystem health. Although the frozen (hydrated) state of the sample can induce considerable self-absorption effects for the low energy fluorescent lines (e.g. Ca-K_α), these effects were of minor importance for higher Z elements for this particular sample [2].

In this contribution we compared the uptake and distribution of Zn between two *D. magna* genotypes (clones) with different Zn tolerance. Based on ecotoxicological experiments, in which reproductive performance of both genotypes was recorded under a control and a Zn exposure (250 µg/L), clone 'O22' showed low tolerance to Zn while clone 'M27' revealed high tolerance to Zn. All samples (i.e. *Daphnia magna* individuals) harvested for the present study, were exposed for 7 days to the same control medium and also to the same 250 µg/L Zn concentration.

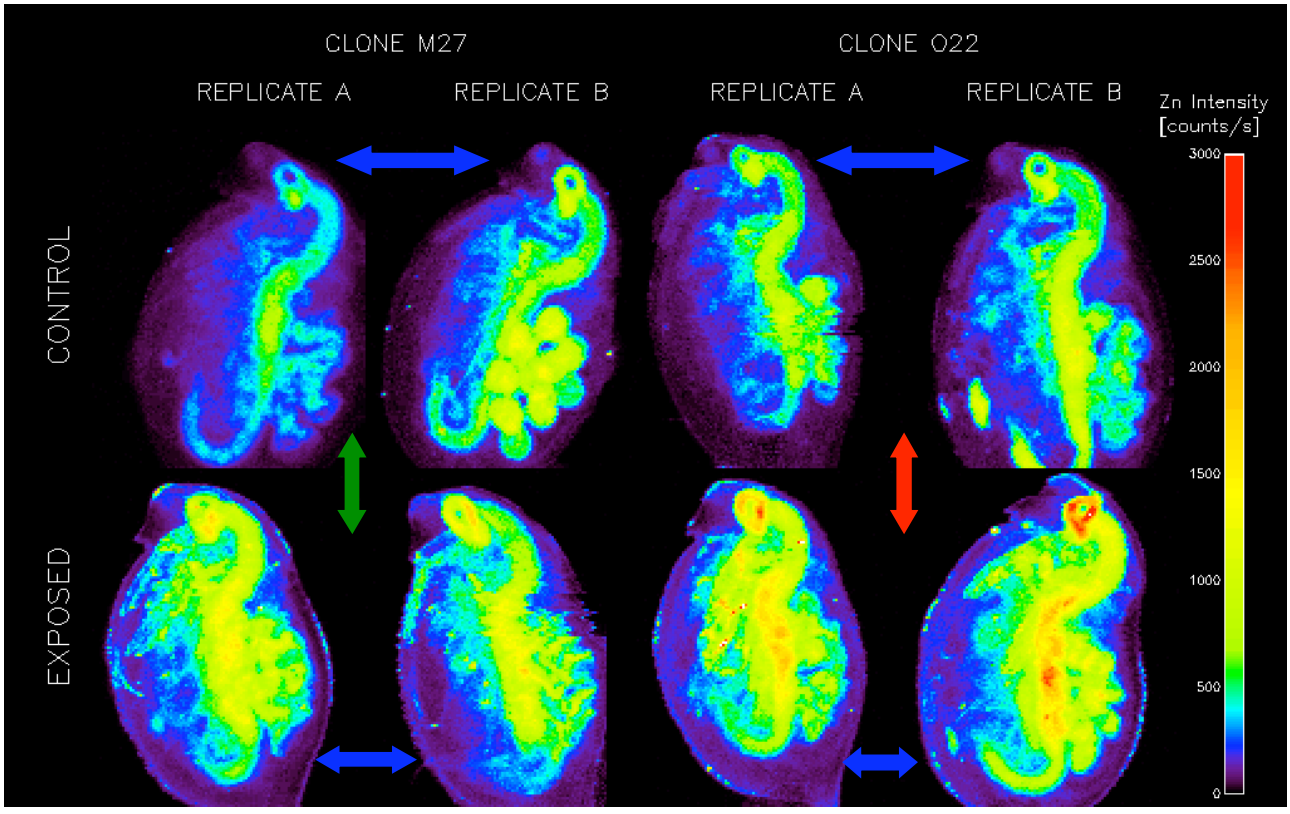

Figure 1: Zn distributions of *Daphnia magna* (M27 and O22) with different Zn tolerance under different exposure conditions (control medium and 250 µg/L Zn) obtained under cryogenic conditions.

Fig. 1 shows the Zn elemental distributions within the two *Daphnia magna* genotypes. Per exposure condition and per genotype, two replicates were measured (replicate A and replicate B). Individual point spectra were collected from both SDD detectors by means of a dynamic scanning 2D micro-XRF scan. All individual element maps were scaled with respect to the same colour bar, indicating the Zn intensity in counts/s. A histogram of the dataset was used to remove hot spots from the element maps, to ensure an adequate scaling and comparison of the samples.

The variation in Zn distributions between control and exposed samples (indicated by green/red arrow) is higher than the variation between replicates within the same exposure condition (indicated by blue arrows). A higher Zn concentration can be observed in the region of gut, eggs, gill tissue and digestive gland of the Zn exposed *Daphnia* compared to those from the control medium. Interestingly, the less Zn tolerant clone 'O22' showed a higher Zn accumulation in these tissues when exposed to 250 μg/L of Zn (red arrow) as compared to the more Zn tolerant clone 'M22' (green arrow). This is a first indication that genetically determined differences in Zn tolerance may be related to differences in Zn accumulation (e.g. through higher Zn uptake rates or lower Zn elimination rates in less Zn tolerant genotypes). Further research with more genotypes is underway.

References

[1] B. De Samber et al., Cryomapping on Daphnia magna at Beamline L using a cryostream: experimental setup and comparison, Hasylab Annual Report 2008

[2] B. De Samber et al., Dual Detector micro-XRF Cryotomography and Mapping on the Model Organism Daphnia magna, Hasylab Annual Report 2009 (in press).