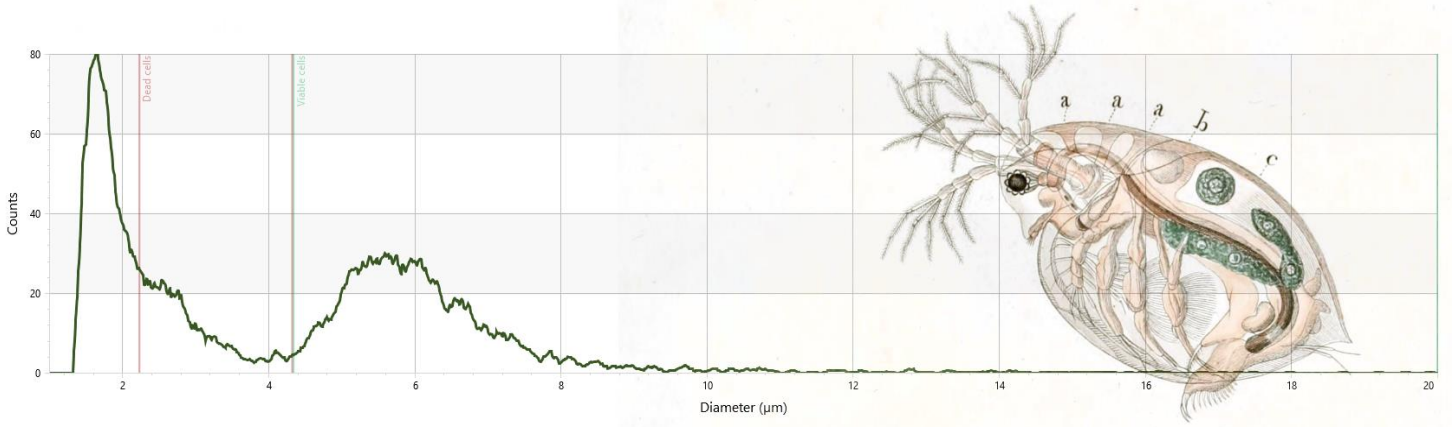
OMNI Life Science

Application Note

Ecotoxicology: Algae Feeding Rates of *Daphnia magna*

Evaluation of an important marker of animal health with CASY



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Introduction

Phenotypic markers of animal health form an essential component of regulatory toxicology. The water fleas - *Daphnia magna* – provide a key species for freshwater ecotoxicology. An important marker of animal physiology and health is feeding rate. Developing miniaturised approaches would use fewer animals, less media and chemicals, less laboratory space and make the tests more compatible with automation, and therefore could result in considerable time savings. Furthermore, miniaturising phenotypic markers to the ultimate level of a single animal per well would facilitate multiple measurements of other phenotypic markers, such as behavioural responses, which could be integrated at the individual level.

Using a CASY cell counter we developed a multiwell plate test that produces equivalent results as for traditional test configurations, for different chemicals.

Methods

Having studied the impacts of varying algae concentration, total volume and animal density, we used this miniaturised test to show the impact of metals on the feeding activity on daphniids.

Daphniids are exposed to chemicals or pollutants for a defined exposure period. An algal suspension (Chlorella or Chlamydomonas species) is prepared and its concentration is measured using the CASY cell counter.

Daphniids are transferred into well plates with the same fixed volume of algal suspension.

After a feeding period (usually 1-2 hours), daphniids are removed and the concentration of algae is measured in each well. The consumption of algae or feeding rate is estimated by the difference in algae concentration and expressed per individual (Figure 1).

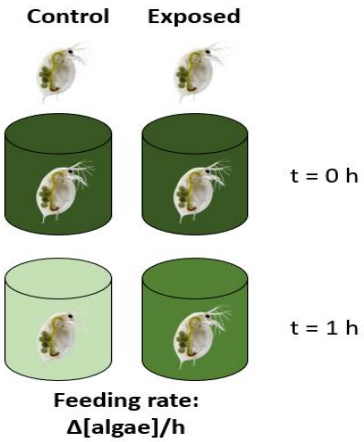

Fig. 1: **Experiment Layout.** Daphniids are transferred into well plates containing a defined amount of algae. After 1 – 2 hours the algae concentration is measured again.

keywords: OECD, Immobilisation, Algae, Multiwell plate, Automation, Metal

Title image: CASY plot of Chlorella vulgaris (OLS) and Illustration by Christine Jurine in Histoire des monocles qui se trouvent aux environs de Genève by Jurine, Louis, 1751-1819, Pl. 8. Published online by Biodiversity Heritage Library. https://doi.org/10.5962/bhl.title.10137

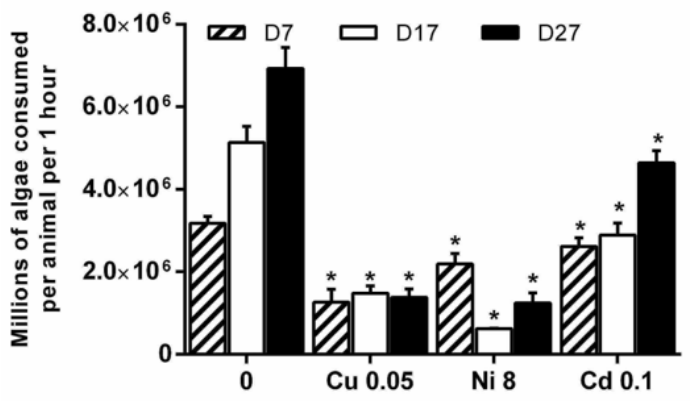

Fig. 2: **Feeding rate** was determined with daphniids of different ages exposed for 24 hours to metal stress and the impact on algae consumption was assessed. Data represent average±SD (n=5). *Statistically significant effect of metal treatment relative to unexposed controls, conducted for each age group (ANOVA, p<0.05). Daphniids age was 7 days (D7), 17 days (D17) and 27 days (D27).

Results

Having standardised our approach, we employed the test system to analyse the impacts of metals on feeding rate (Figure 2).

The control (0) shows that the feeding rate of daphniids clearly correlate with age.

Feeding rate significantly drops after a 24-hour exposure to different metals used in this study: Cu (0.05 mg/l), Ni (8 mg/l), Cd (0.1mg/l).

During growth, the response of daphniids to a 24-hour metal exposure is different for different ages (D7, D17, D27), elder animals showed major changes.

Conclusion

Feeding rate is an important parameter for estimating the allocation of energy supplies in cladocerans and this test might well contribute to **toxicological studies**.

In this study we developed an **optimised, miniaturised** test system for acute toxicity and algae feeding measurements in daphniids.

The feasibility of a **multiwell approach** for *Daphnia* toxicity testing is confirmed. It requires less time and materials than a traditional assay and can provide **phenotypic characterisation** at a single animal level.

The CASY cell counter proved to be an accurate and sensitive instrument for ecotoxicological applications allowing fast, simple and label-free measurements.

References

1. Grintzalis K, Dai W, Panagiotidis K, Belavgeni A, Viant MR. Miniaturising acute toxicity and feeding rate measurements in *Daphnia magna*. Ecotoxicol Environ Saf. 2017, 139:352-357. https://doi.org/10.1016/j.ecoenv.2017.02.002

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