To avoid or not to avoid REE contaminated sediment? That is the question for Daphnia

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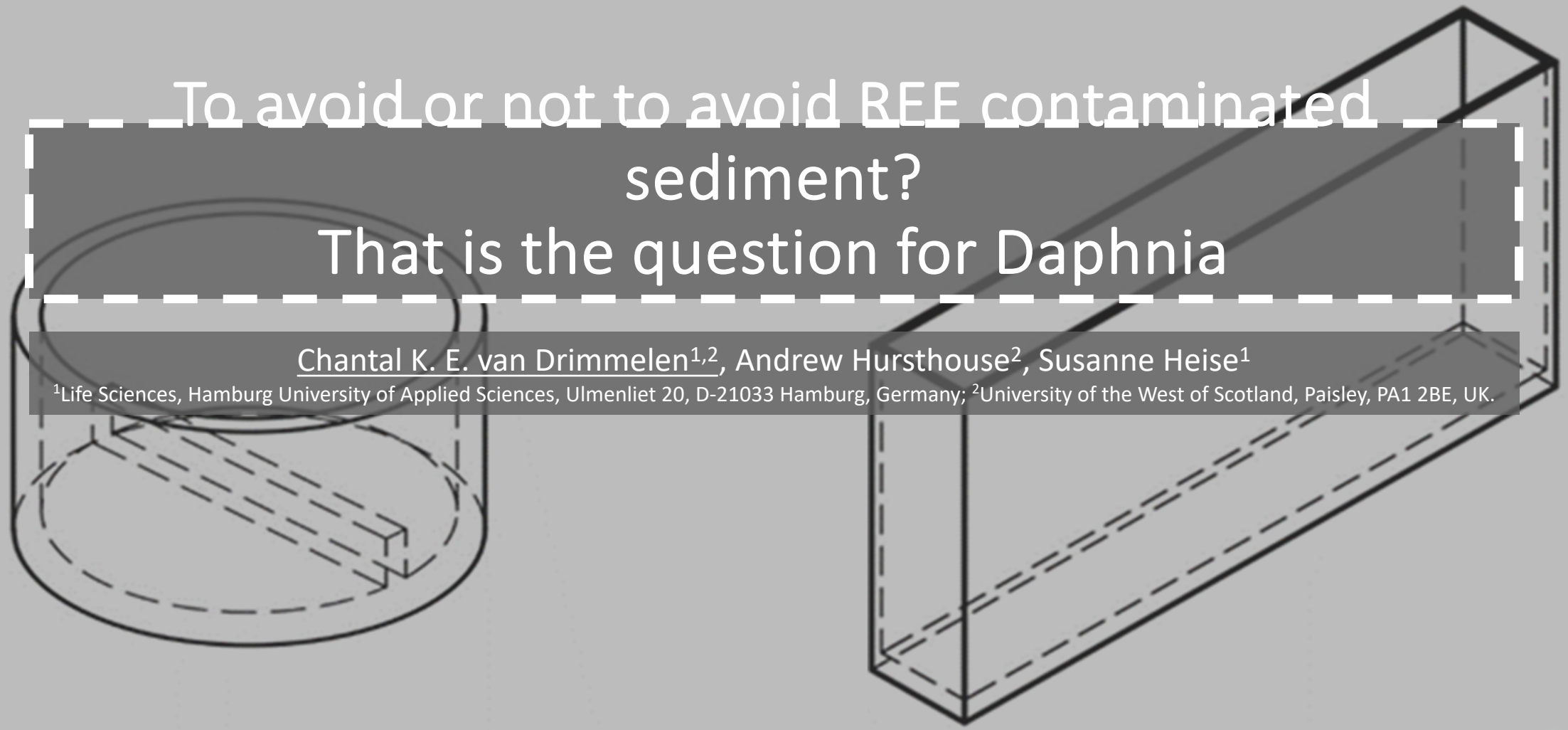


ITN PANORAMA – This project has received funding from European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie Grant Agreement N°857989.

Introduction

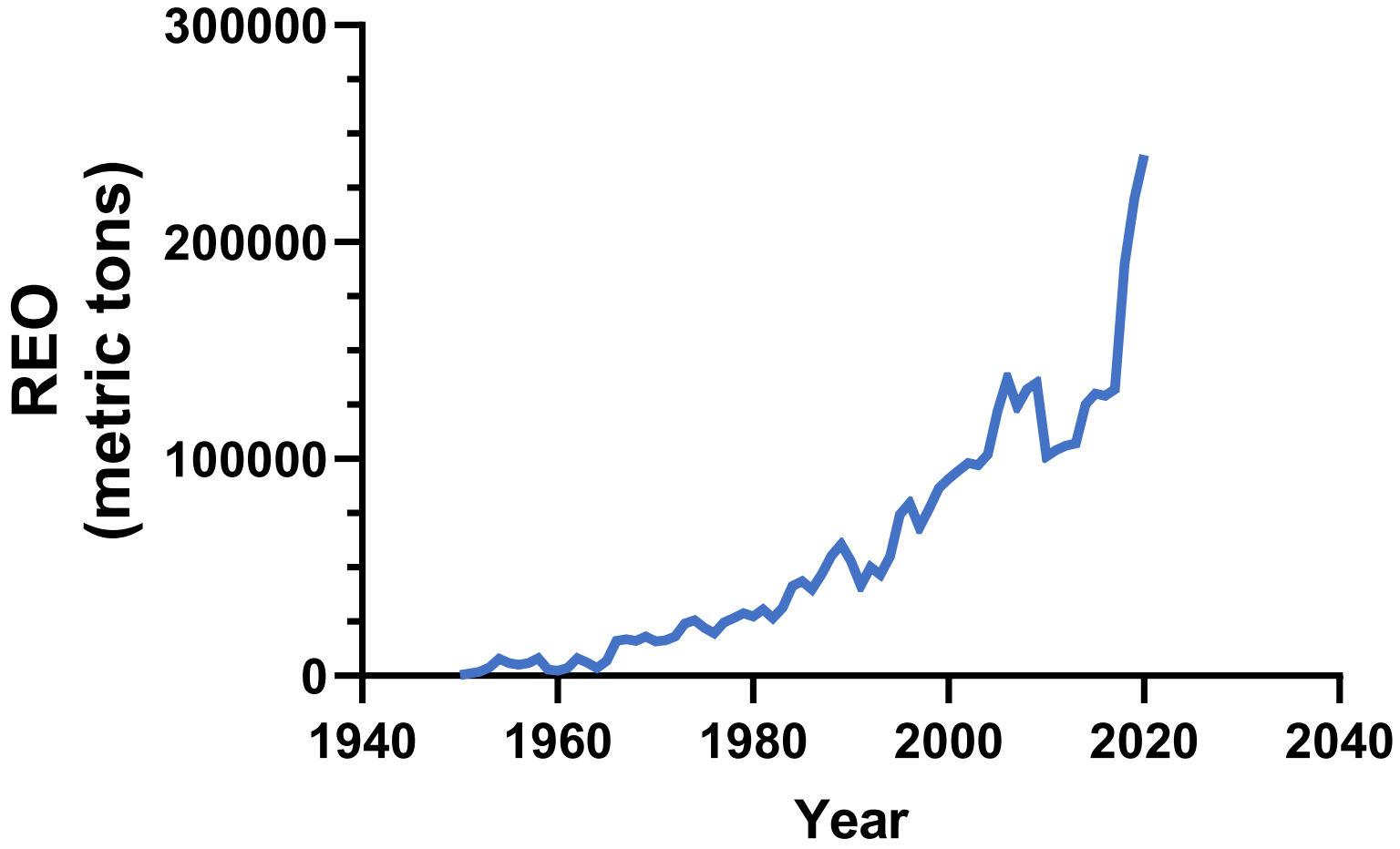
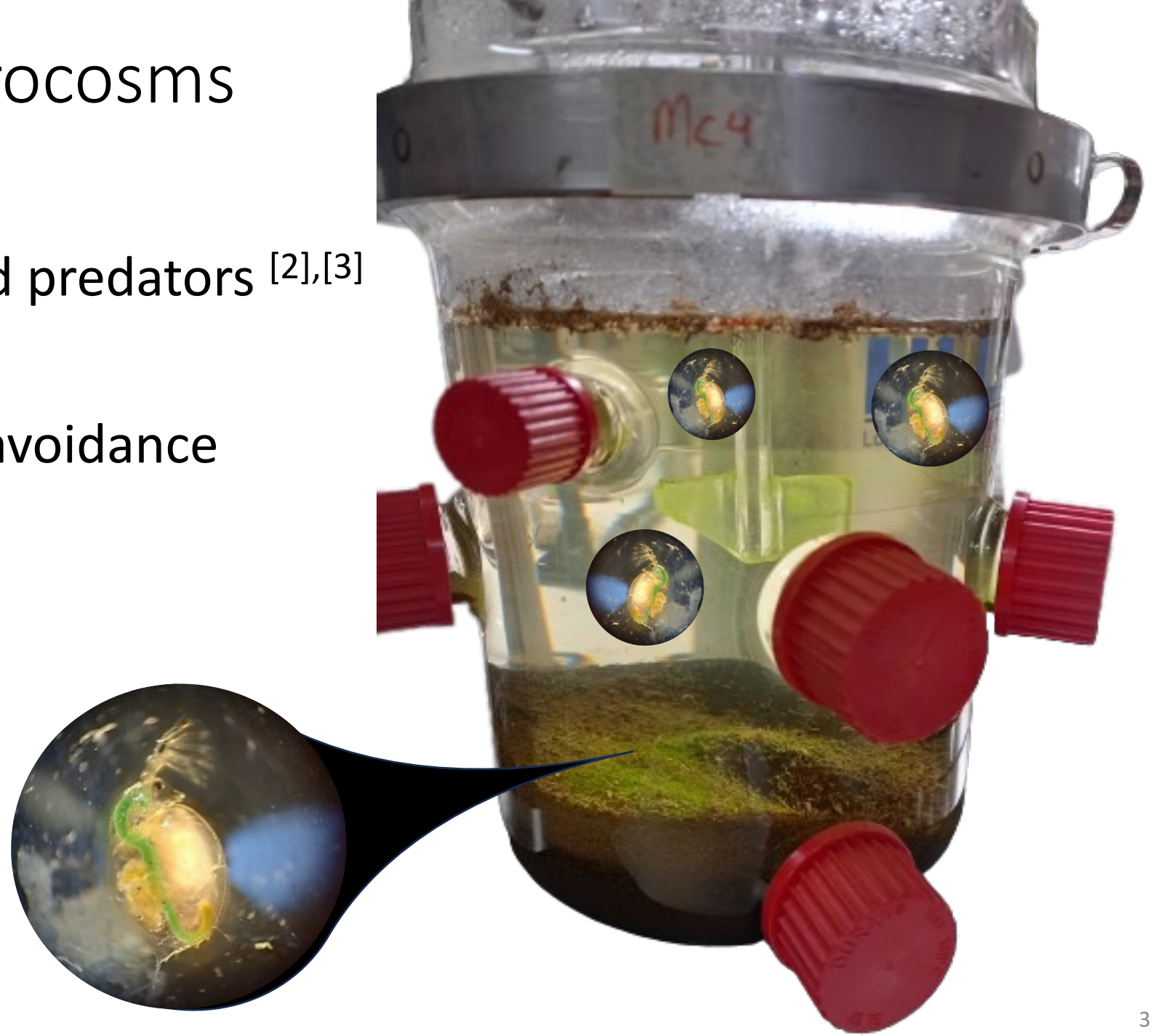


Fig. 1. Global rare-earth-oxide (REO) production in metric tons (1950-2020) [1]

Observations of microcosms

- Vertical migration to avoid predators [2],[3]
- Indirect effects → active avoidance

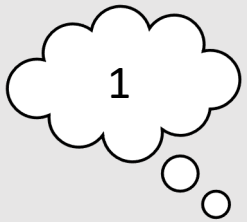


Objective/Hypothesis

Objective:

Determine if lanthanum (La) and gadolinium (Gd) leads to avoidance behaviour by pelagic species

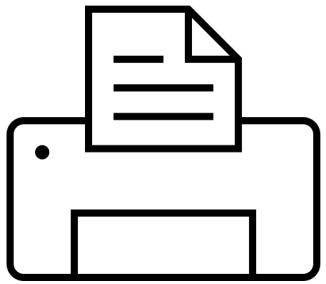
Hypothesis:



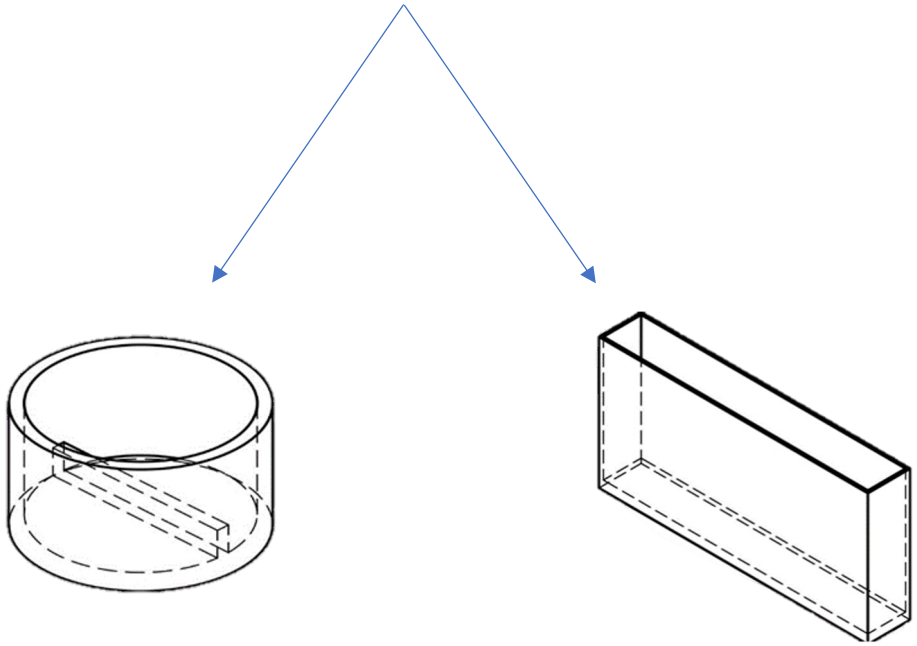
Avoidance behaviour will occur both vertical and horizontal for *Daphnia magna*

Different avoidance behaviour for La and Gd

Materials & Methods



3D print

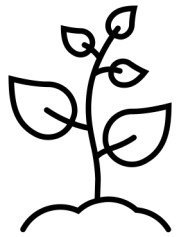


Horizontal

Vertical

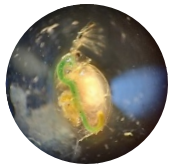
ISO M4 Ø EDTA

+



Lufa 2.2

Anthropogenic concentrations (mg kg^{-1})	Environmental concentrations (mg kg^{-1})
25	0.001
50	0.01
75	0.1
100	1
	10



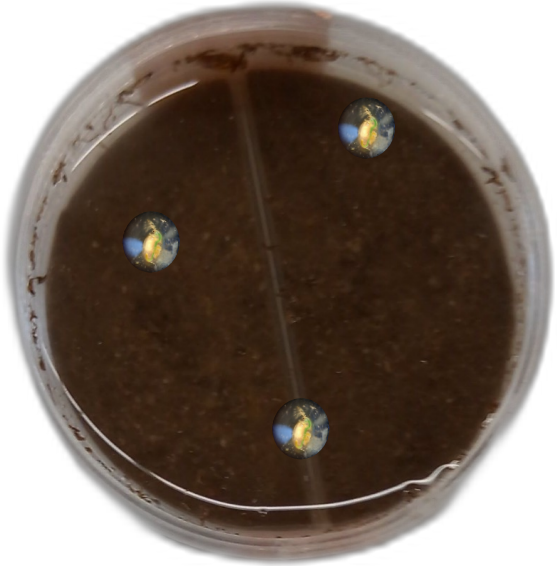
3x *Daphnia magna*

15 sec. → OECD 202

→ 0h, 1h, 2h, and 3h

5 repetitions

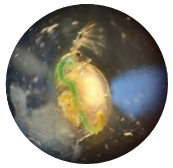
0



1

Horizontal

OECD, *Test No. 202: Daphnia sp. Acute Immobilisation Test*, 2004



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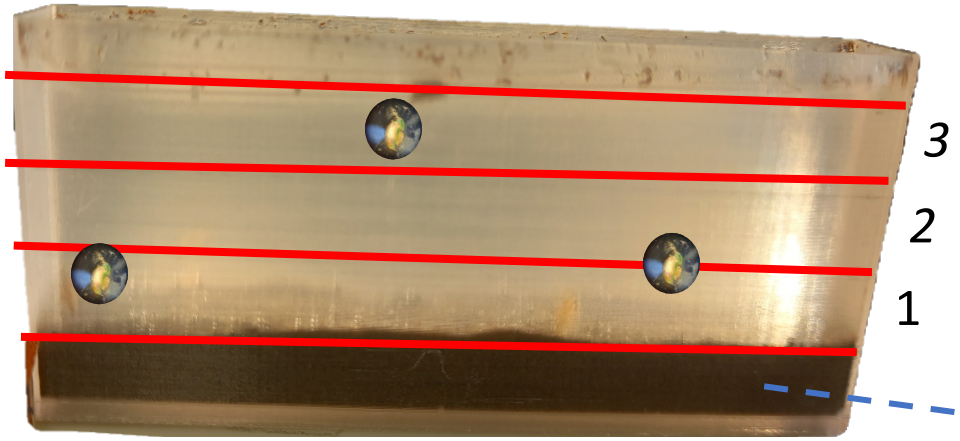


Vertical

or

OECD, *Test No. 202: Daphnia sp. Acute Immobilisation Test*, 2004

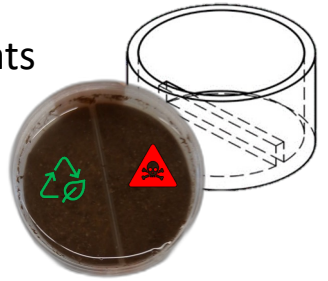
Results

So….

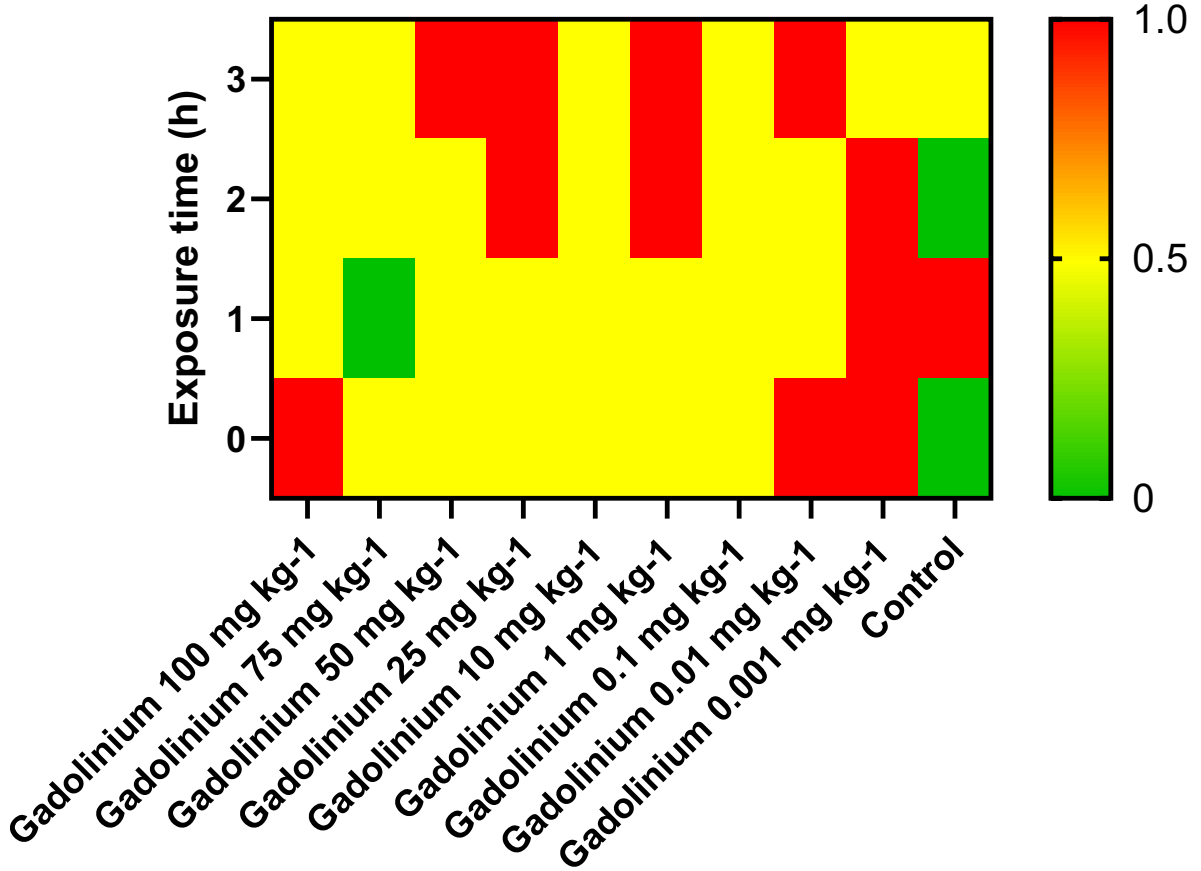
Does REE Contaminated Sediment Cause Avoidance Behaviour of *D. magna?*

Horizontal

3x daphnids, 5 repeats



Average scoring:



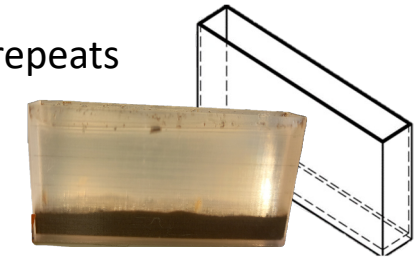
Frequency distribution:

La Chi-square $p > 0.05$

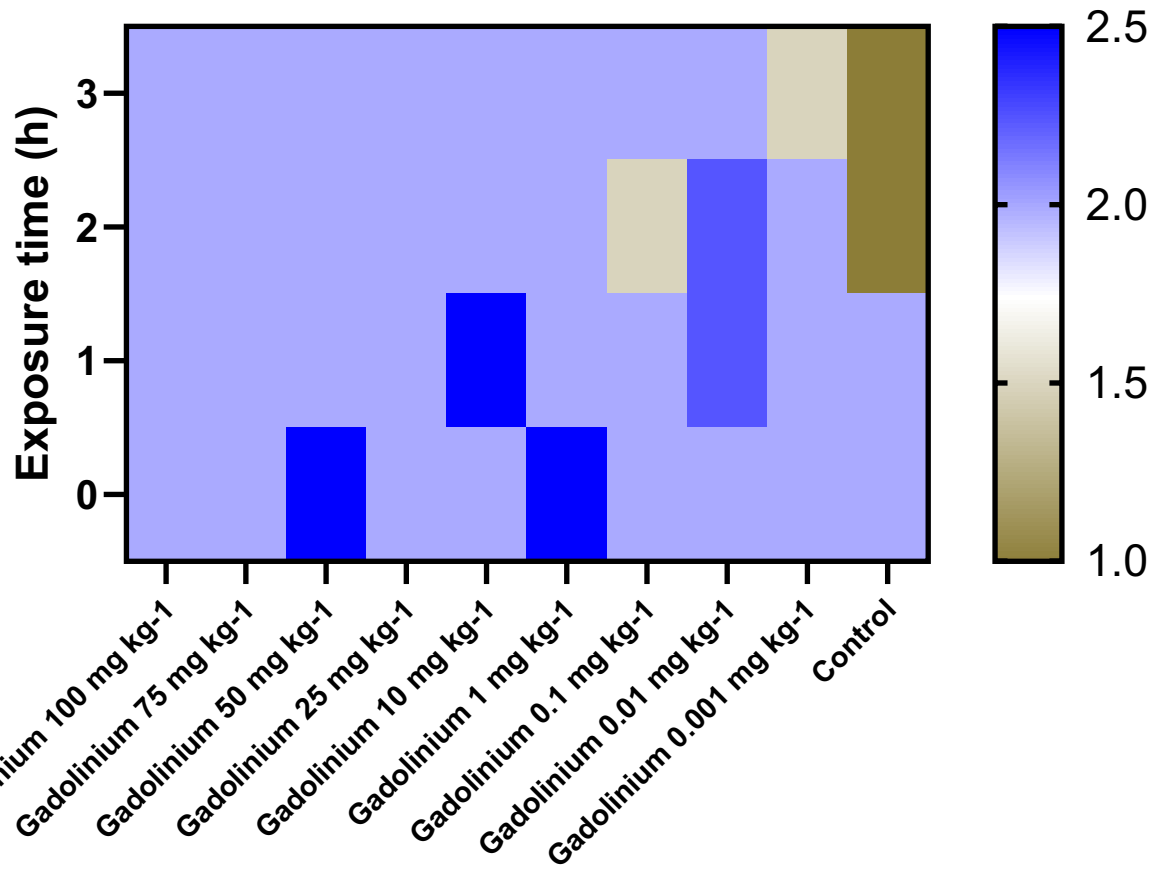
Gd Chi-square $p < 0.05$ ⟶ 50; 1; 0.01; and 0.001 mg kg^{-1}

Vertical

3x daphnids, 5 repeats



Average scoring:



Frequency distribution:

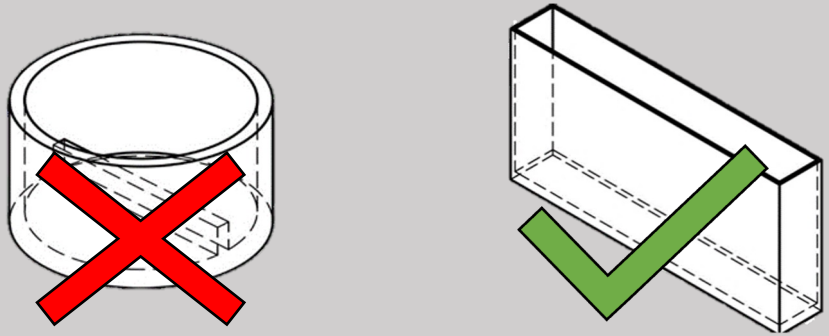
Chi-square $p < 0.01$ →

La not 10; 0.01; and 0.001 mg kg^{-1}
Gd not 0.001 mg kg^{-1}

Results

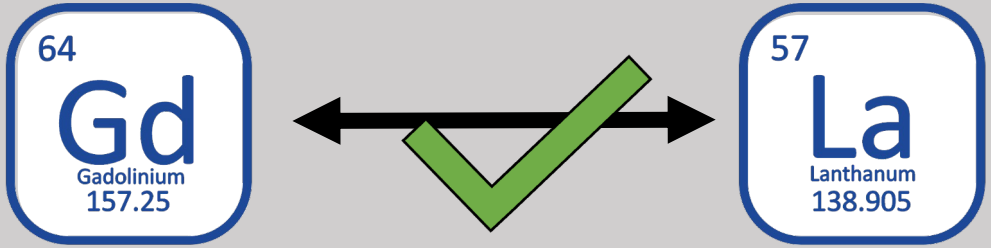
Hypothesis 1:

Avoidance behaviour will occur both horizontal and vertical for *D. magna*



Hypothesis 2:

Different avoidance behaviour for Gd and La



Discussion and Conclusion

- Contaminated REE sediment causes avoidance in *D. magna*
- REE type and concentration impacts avoidance

- Variation REE speciation/partitioning → but complex and knowledge lacking [4]

Discussion and Conclusion

- Daphnia can sense natural chemical signals [6] → also the case for REE?

- Avoidance more sensitive endpoint than mortality [5]
- Perceived stress → behavioural changes
- Behavioural changes → More susceptible to predators [7]

- Behavioural studies could be an issue for future risk assessment

Take home message & Remaining questions

Behaviour should be tested in various ways

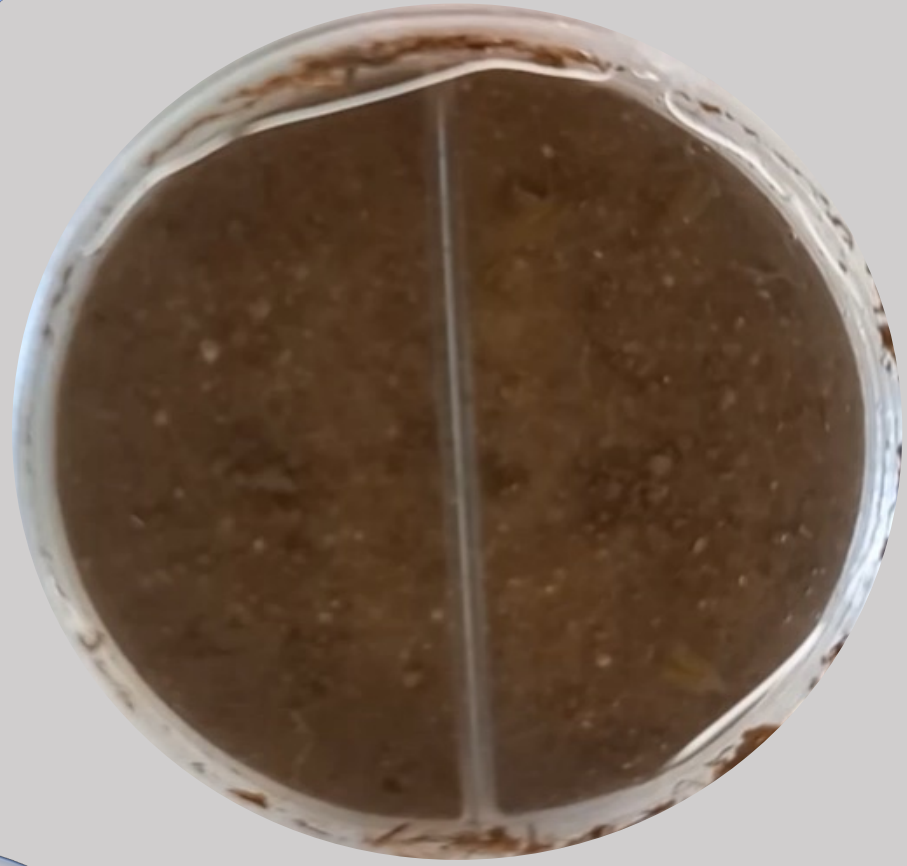
→ e.g., Mobility and avoidance

Behaviour changes can have an environmental significance

→ consequence for risk assessment

→ standardization

More need to consider bentho-pelagic interaction



THANK YOU!

Also many thanks to Lucas Janssen Nieto
and Florian Stukenkemper!

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References

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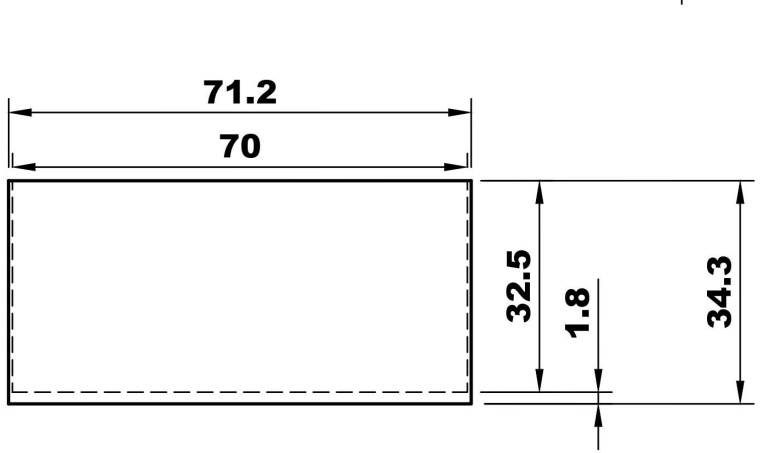

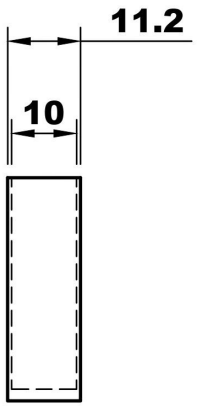

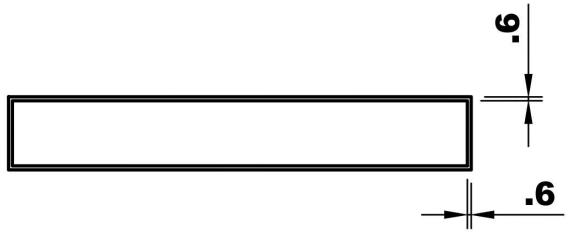

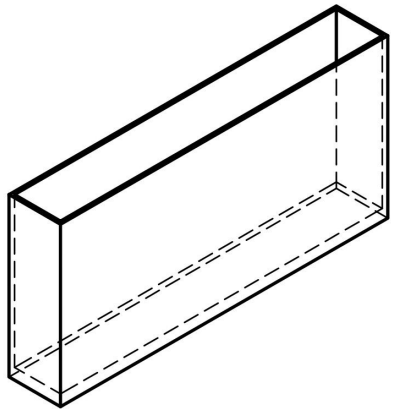

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		Document type: Technische Zeichnung	Document status: Abgeschlossen
		Title: Daphnientest Vertikal	DWG No.: 2
			Rev.: 1 / Date of issue: 20/03/2023 / Sheet: 1/1

PETG Filament

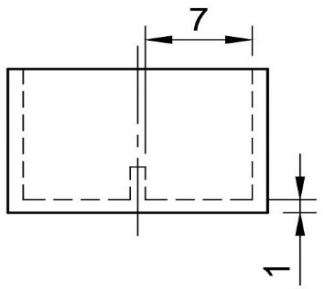
Vertical shells:
Perimeters: 1

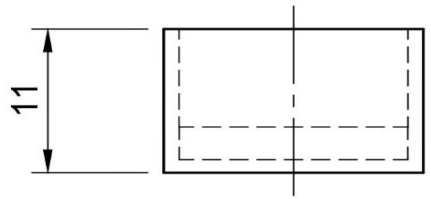
Horizontal shells:
Top and Bottom = 0

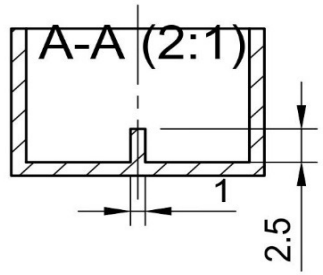
Infill:
100% Aligned Rectilinear
2,5% infill anchor

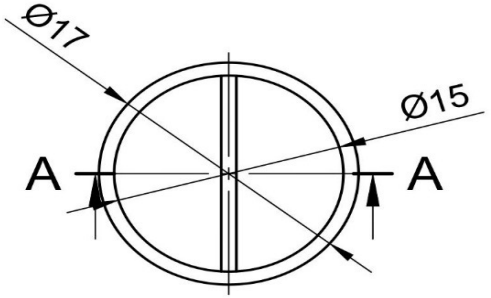
Speed:
Everything to 15 mm/s

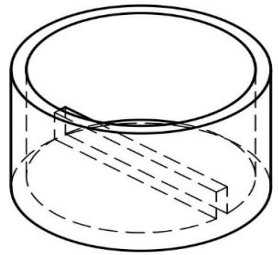
Extrusion multiplier: 1,025
Temp:
Nozzle: 230 °C
Bed: 70°C
Fan Speed: 0%











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			Rev. / Date of issue / Sheet: 1/1

Chemical and physical characteristics of standard soils according to GLP

LUFA Speyer
Obere Langgasse 40
67346 Speyer
Tel.: 0 62 32/136-0
Fax: 0 62 32/136-110
Mail: info@lufa-speyer.de

LUFA Speyer is an agricultural institution of Bezirksverband Pfalz

(Mean values of different batch analyses +- standard deviation. All values refer to dry matter.)

Standard soil type no.	2.1	2.2	2.3	2.4	5M	6S
Batch No. (Sp=stored; F= field fresh)						
Sampling date						
Organic carbon (% C)	0.61+- 0.05	1.77 +- 0.56	0.66 +- 0.09	1.83 - 0.25	0.88 +- 0.18	1.55 +- 0.14
Nitrogen (% N)	0.06 +- 0.02	0.20 +- 0.06	0.08 +- 0.02	0.23 +- 0.02	0.11 +- 0.03	0.18 +- 0.01
pH value (0.01 M $CaCl_2$)	4.7+- 0.1	5.6 +- 0.3	6.2 +- 0.3	7.5 +- 0.1	7.4 +- 0.1	7.3 +- 0.1
Cation exchange capacity (meq/100g)	3.1 +- 0.4	8.5 +- 2.0	6.0 +- 0.9	17.6 +- 1.0	8.5 +- 0.25	18.7 +- 1.2
Particle size distribution (mm) according to German DIN (%):						
<0.002	3.9 +- 0.8	10.6 +- 1.9	7.4 +- 0.9	24,5 +- 1.8	11.9 +- 1.0	41.9 +- 2.7
0.002 - 0.006	1.8 +- 0.7	3.3 +- 1.1	4.8 +- 0.5	7.8 +- 0.6	5.4 +- 0.9	9.9 +- 0.8
0.006 - 0.02	3.0 +- 0.4	5.3 +- 0.5	10.9 +- 0.9	14.8 +- 1.2	9.2 +- 0.9	11.8 +- 0.9
0.02 - 0.063	5.0 +- 1.0	7.4 +- 0.9	19.4 +- 0.7	25.3 +- 2.5	21.8 +- 1.9	14.9 +- 1.1
0.063 - 0.2	29.2 +- 1.5	30.9 +- 3.4	26.2 +- 0.8	20.4 +- 1.1	37.7 +- 2.6	10.0 +- 0.8
0.2 - 0.63	54.7 +- 1.5	41.6 +- 3.0	29.0 +- 1.4	5.6 +- 1.8	12.8 +- 1.5	9.3 +- 1.3
0.63 - 2.0	2.3 +- 0.3	0.9 +- 0.1	2.4 +- 0.2	1.5 +- 0.4	1.2 +- 0.5	2.2 +- 0.4
Soil type	sand (sS)	loamy sand (lS)	silty sand (uS)	sandy loam (sL)	loamy sand (lS)	clayey loam (tL)
Particle size distribution (mm) according to USDA (%):						
<0.002	3.9 +- 0.8	10.6 +- 1.9	7.4 +- 0.9	24.5 +- 1.8	11.9 +- 1.0	41.9 +- 2.7
0.002 - 0.05	8.7 +- 1.1	15.0 +- 1.2	33.1 +- 0.8	42.9 +- 1.35	31.6 +- 3.2	35.1 +-0.5
0.05 - 2.0	87.5 +- 1.3	74.4 +- 2.7	59.5 +- 0.7	32.6 +-2.0	56.5 +- 3.3	23.0 +- 2.4
Soil type	loamy sand	sandy loam	sandy loam	loam	sandy loam	clay
Maximum water holding capacity (g/100g)	31.4 +- 2.9	43.3 +- 5.1	35.7+- 2.3	45.6 +- 2.7	41.8 +- 5.3	41.4 +- 1.5
Weight per volume (g/1000ml)	1435 +- 53	1224 +- 103	1302 +- 49	1206+- 58	1219 +- 88	1291 +- 47

(M. Prigge - Phone: +49 (0) 6232 136 125; email: prigge@lufa-speyer.de)

By order of:

Date, signature:

Version 3 D12-18
03.09.2020

p <0.05, Frequency distributions

mg kg^{-1}	**VDRAB Gd**	**VDRAB La**	**HDRAB Gd**	**HDRAB La**
100	<0.0001	0.0014	0.2687	0.4310
75	<0.0001	<0.0001	0.6180	0.1506
50	0.0003	0.0228	0.0116	0.0320
25	0.0008	0.0206	0.1682	0.1832
10	<0.0001	0.2300	0.1039	0.3658
1	<0.0001	0.0095	0.0158	0.6407
0.1	0.0037	0.0306	0.1199	0.0332
0.01	<0.0001	0.3069	0.0168	0.3425
0.001	0.0643	0.0791	0.0341	0.5350
All concentrations	<0.0001	0.0001	0.0185	0.1241

Chronic Daphnia Test

- La and Gd biodistribution differtly

But...
Do the Daphnids notice?

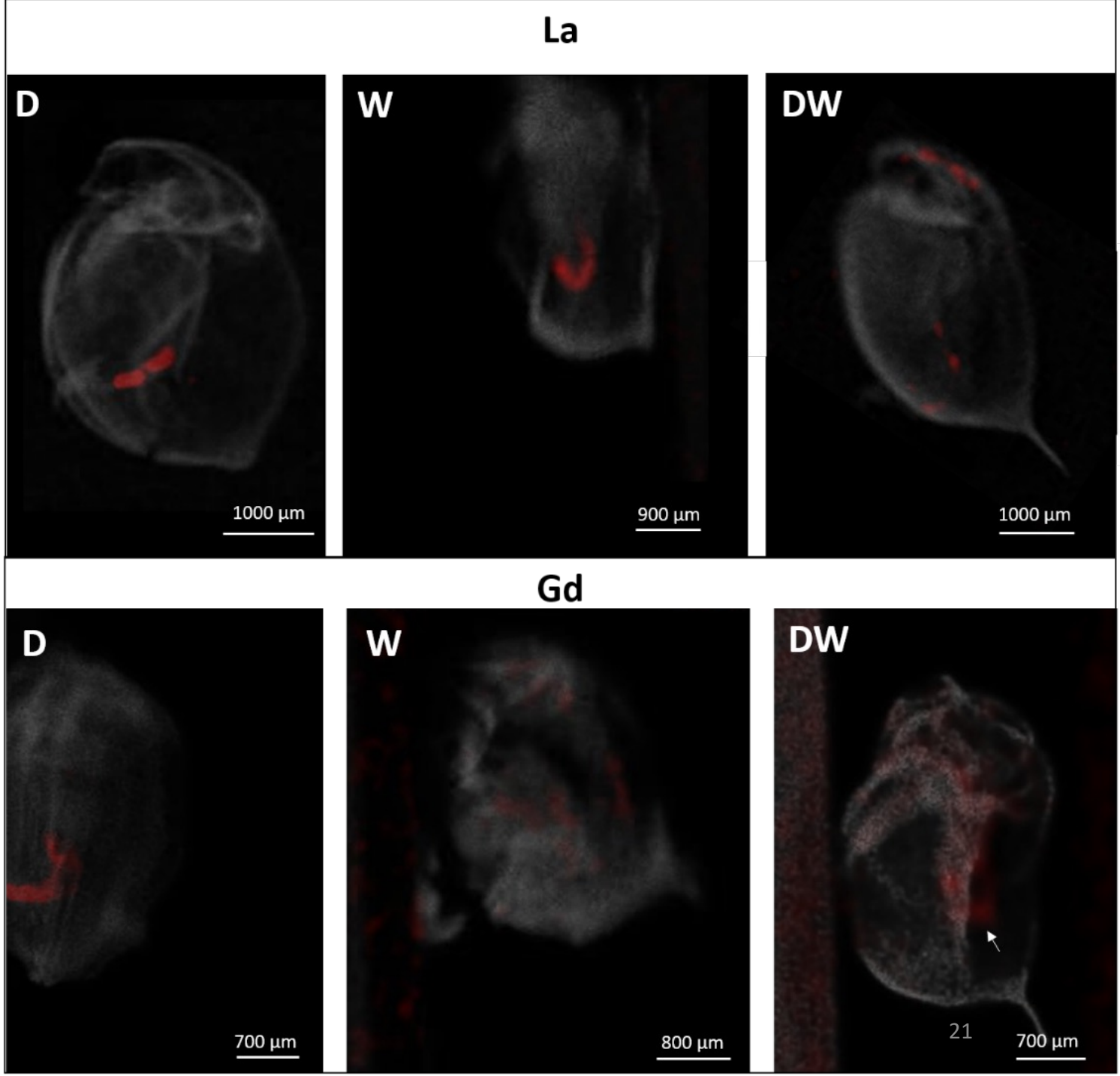


Fig.S1 Lanthanum distribution in 21-day-old Daphnia magna according XRF measurements. Grey: calcium, red: lanthanum. D: Dietary exposure, W: waterborne exposure and DW: Dietary and Waterborne exposure. (Revel et al. 2023, Submitted)

“Co-localisation of Gd with S and P suggests that Gd accumulates in the tissues, such as the gills, maxillary glands and possibly a part of the intestinal tract (ESI data S4†).(Revel et al., 2023)

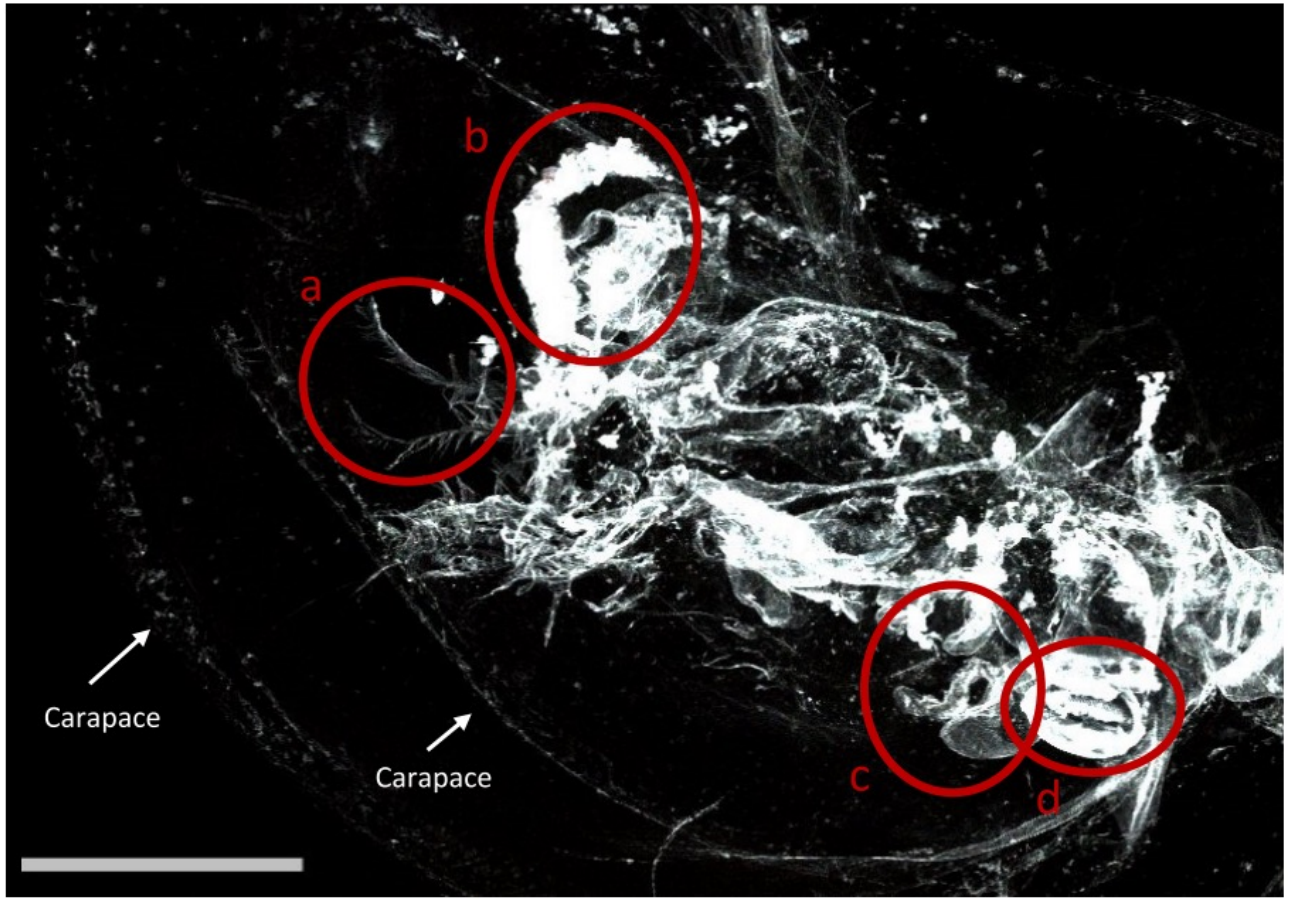
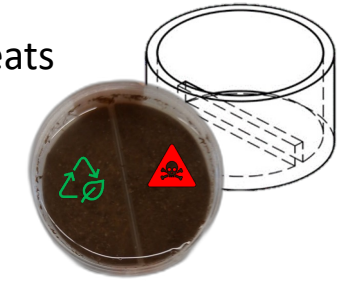


Fig.S2 Distribution of Gd in organism of figure 2e (exposed to 15mg L-1 of Gd for 48h). Area 13 selected: a. Filtering setae, b. intestine, c. shell gland, d. articulation of the antenna. Scale: 200 14 µm. Beamline: NANOSCOPIUM. Incident energy of 17.02 keV, pixel size of 1 µm, 15 integration time of 20 ms. (Revel et al., 2023)

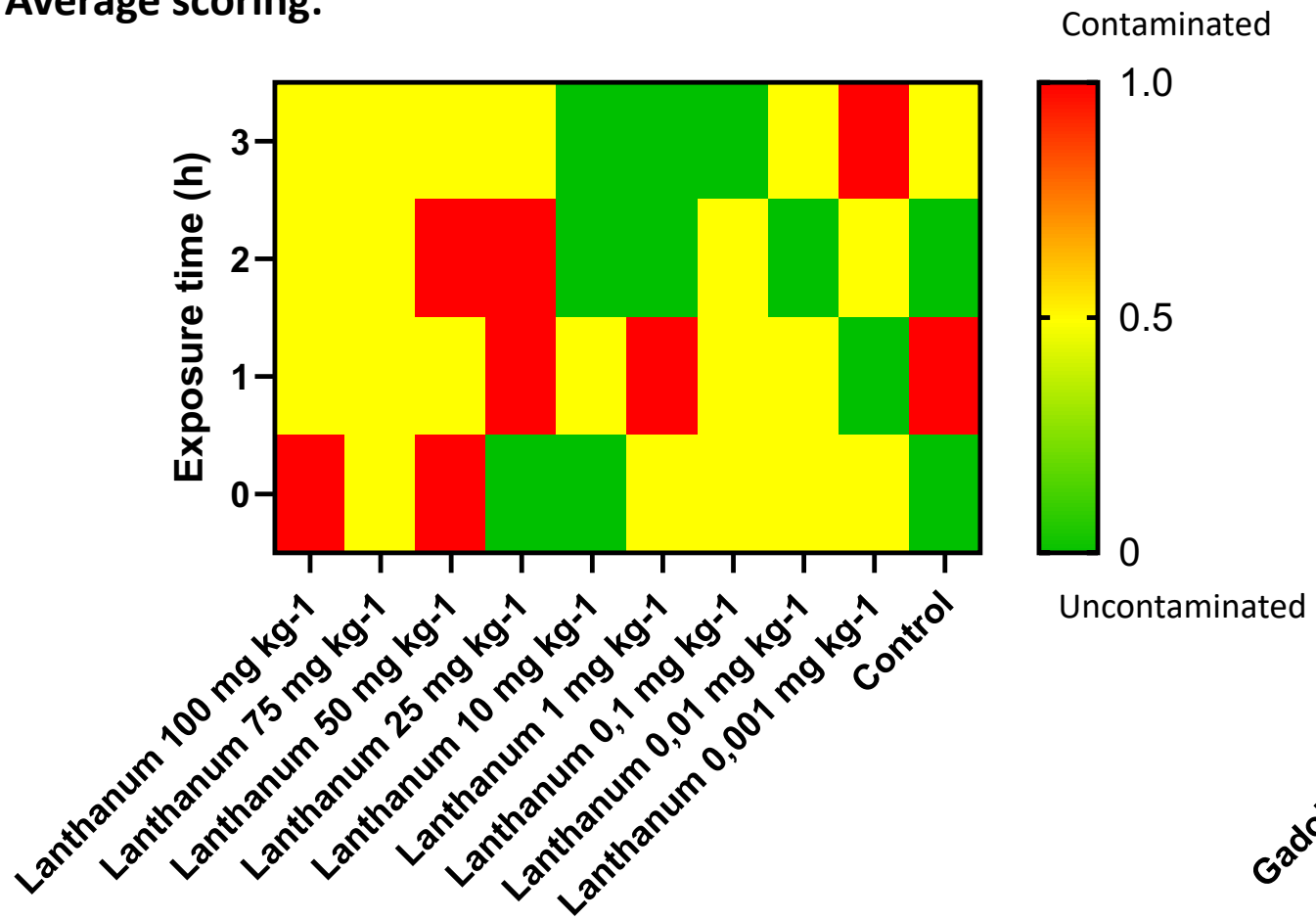
Revel, M., et al., *Determination of the distribution of rare earth elements La and Gd in Daphnia magna via micro and nano-SXRF imaging.* Environ Sci Process Impacts, 2023.

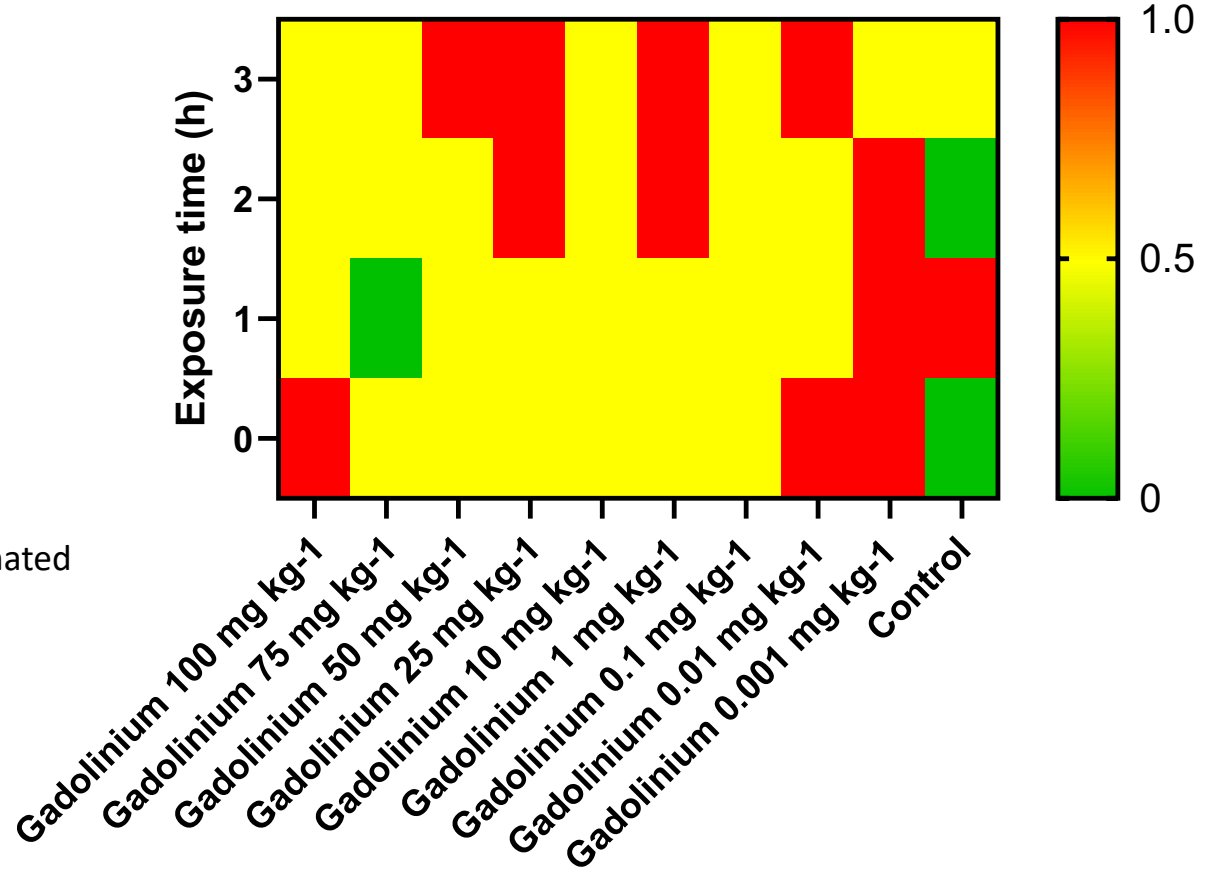
Horizontal

3x daphnids, 5 repeats



Average scoring:





Frequency distribution:

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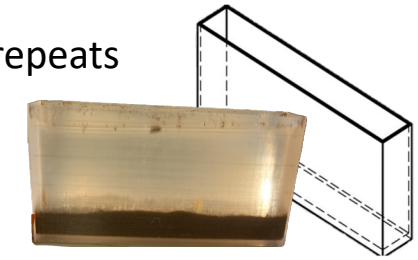
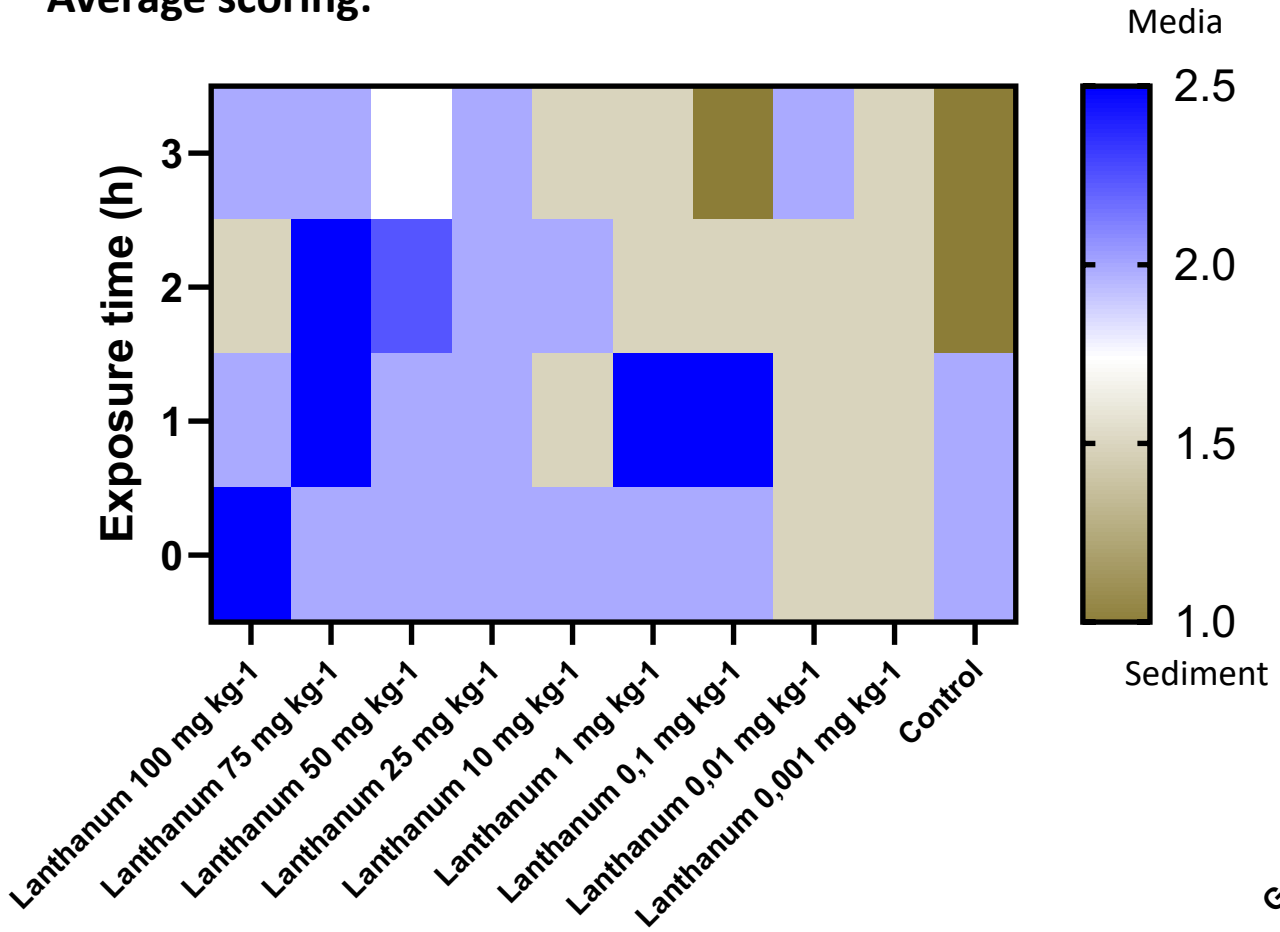
Gd Chi-square $p < 0.05$ ⟶ 50; 1; 0.01; and 0.001 mg kg^{-1}

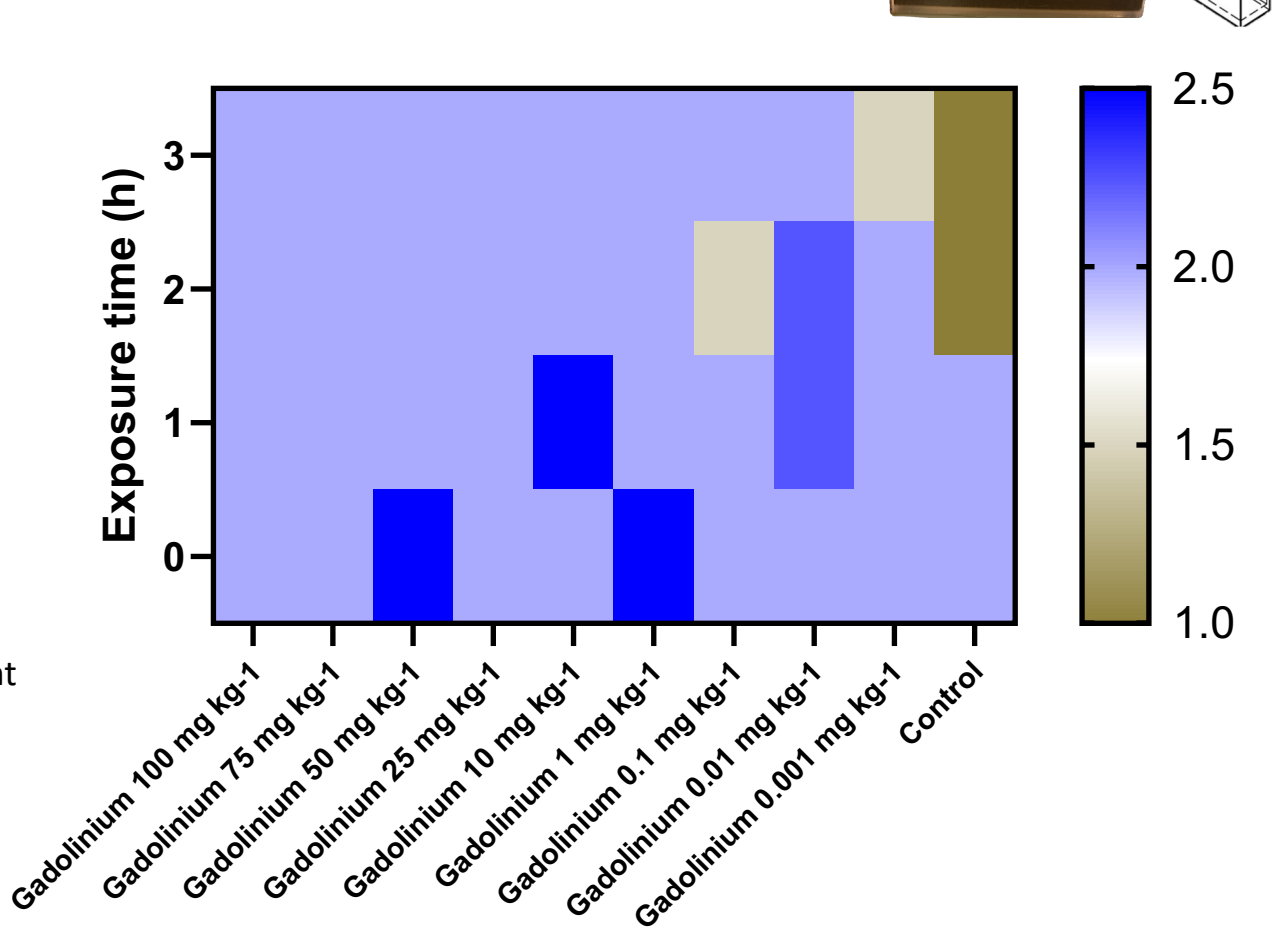
Vertical

3x daphnids, 5 repeats



Average scoring:





Frequency distribution:

Chi-square $p < 0.01$ →

La not 10; 0.01; and 0.001 mg kg^{-1}

Gd not 0.001 mg kg^{-1}