New Insights Into Cadmium Sub-Lethal Effects on *Daphnia magna*

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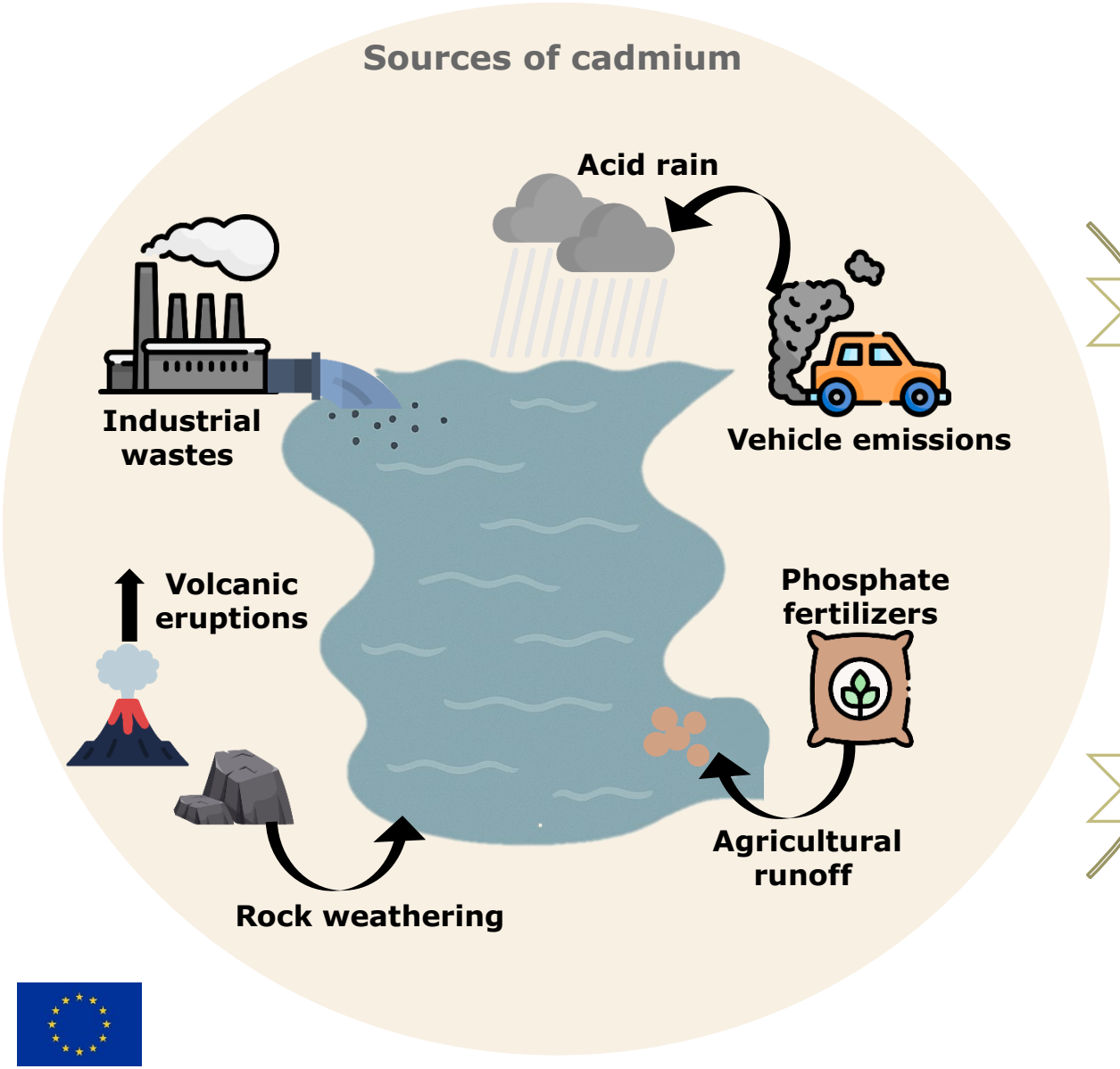

Cadmium in freshwater ecosystems

- Cadmium (Cd) is a global freshwater contaminant
- Higher Cd usage increases Cd occurrence in freshwaters
- Cd is usually found in nature at concentrations bellow 1.25 µg L^{-1}
- Cd full toxicity spectrum towards aquatic biota is unknown

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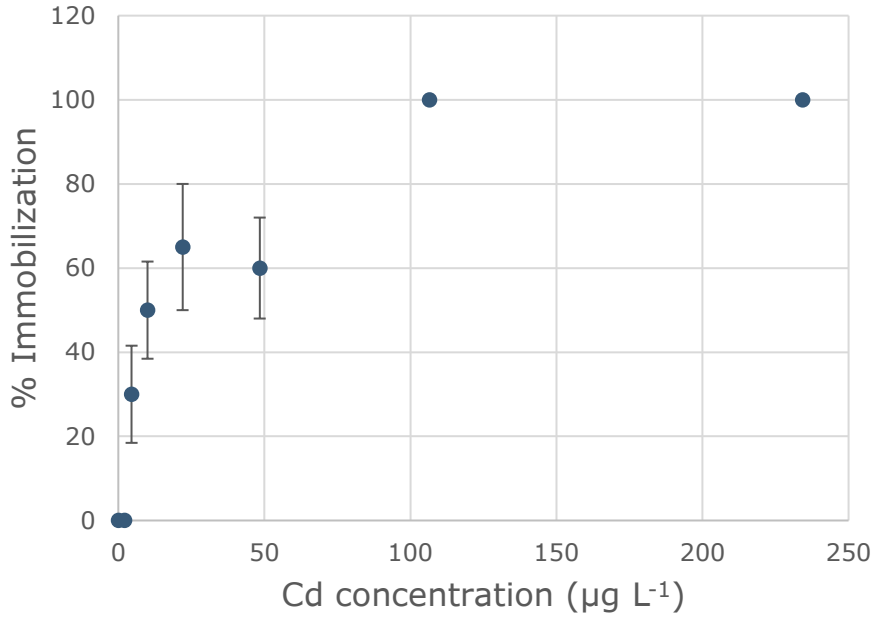
Key objective

Characterize the effects of an environmentally-plausible Cd exposure in *Daphnia magna,* linking molecular responses to downstream phenotypic effects

Methods

1 Acute immobilization test (OECD Test No. 202)



- 48h immobilization data used to calculate the EC20 through Probit analysis
- **EC_{20} = 4.527** (1.718 – 7.790) **µg L^{-1}**

2 Final exposure design

Control

Exposed (EC_{20})

16 h light / 8 h dark

20º C

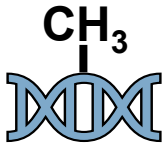
- Exposed from birth to 7 days-old with food supply
- 5 biological replicates

Environmental relevance

Cd concentrations found in a contaminated European freshwater system

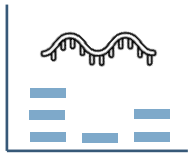
Up to **37 µg L^{-1}** (Bervoets et. al, 2009)

3 Endpoints analyzed



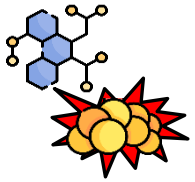
- **Epigenetic biomarkers**

Total DNA methylation (5-mC) levels & Total DNA methyltransferases activity (ELISA kits)



- **Gene expression analysis**

RNA-Seq & Differential gene expression analysis
(GO and KEGG enrichment analysis)



- **Biochemical biomarkers**

Antioxidant enzymes activity and lipid peroxidation levels

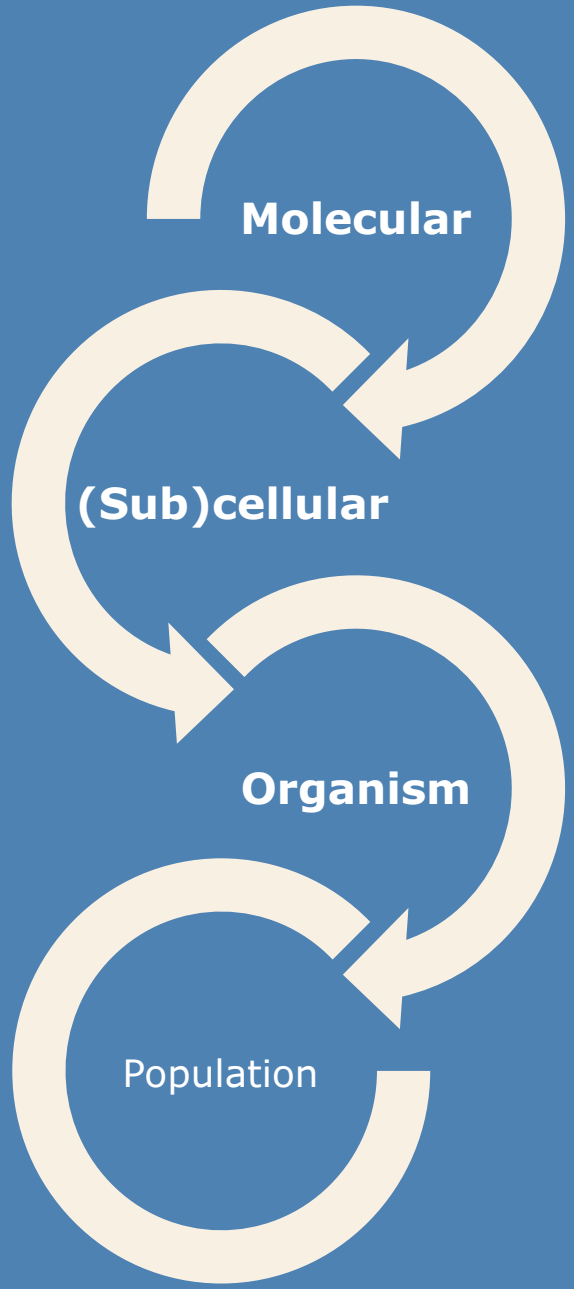
- **Genotoxicity biomarker**

DNA damage levels (Alkaline comet assay)

- **Physiological biomarkers**

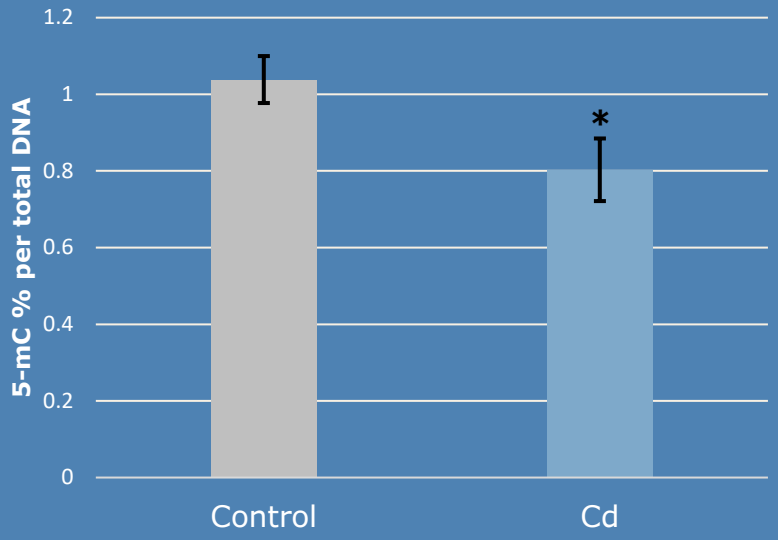
Post-exposure heart rate & somatic growth determination

Levels of biological organization

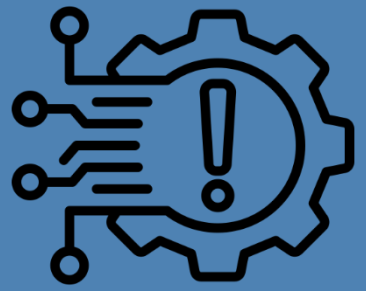


Results & Discussion

Molecular level - Epigenome



➢ Cd exposure alters the epigenetic landscape



“Writer” enzymes ?

DNMTs

Causes

Total DNA methylation

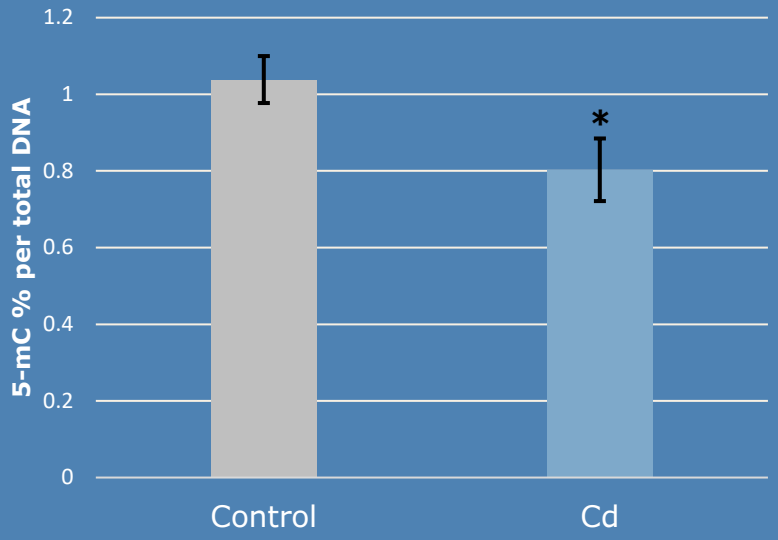
DNMTs activity

Regulators of DNMT activity

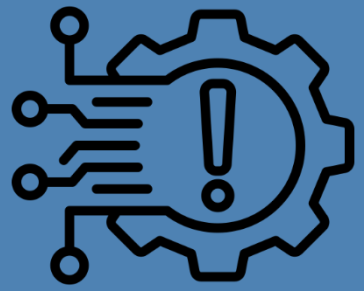
- Alternative splicing events
- Post-translational modifications
- Interaction with other proteins

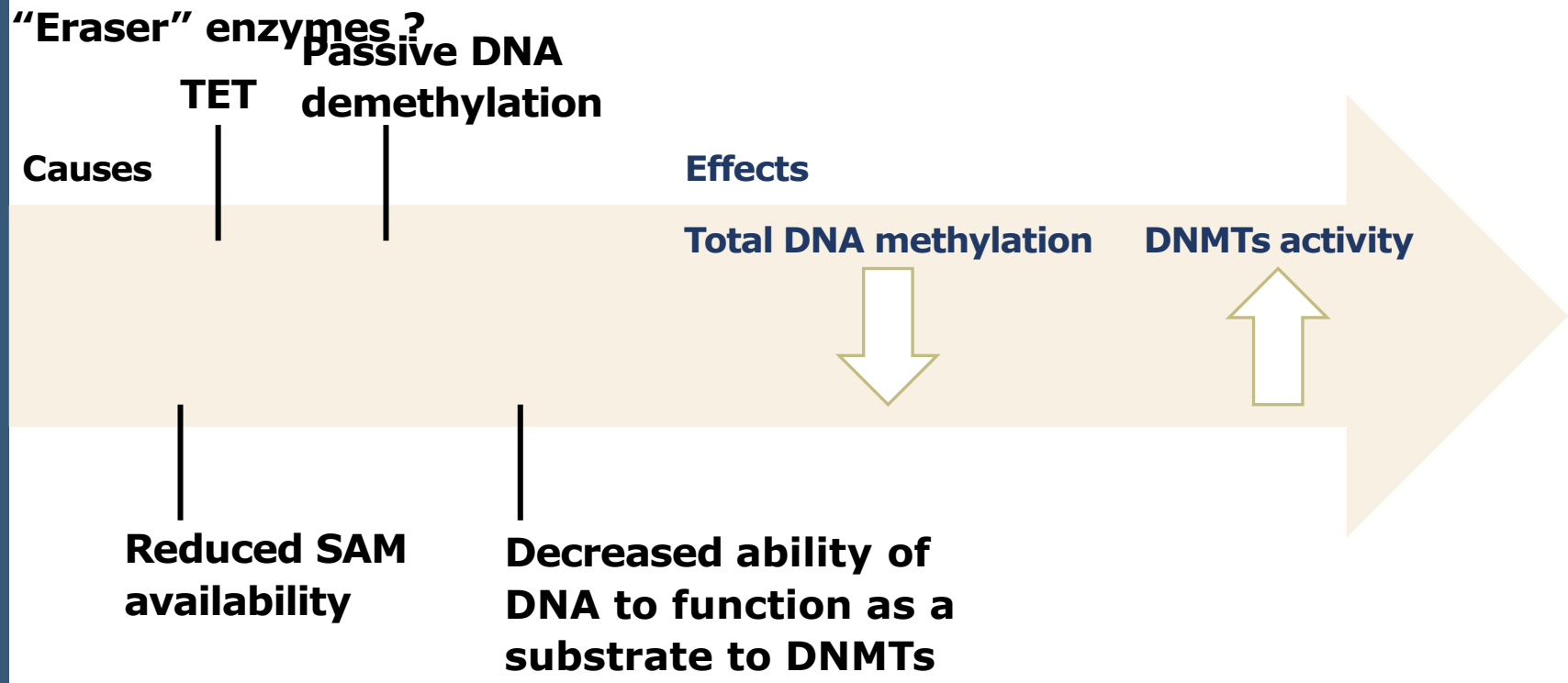
Results & Discussion

Molecular level - Epigenome



- Cd exposure alters the epigenetic landscape

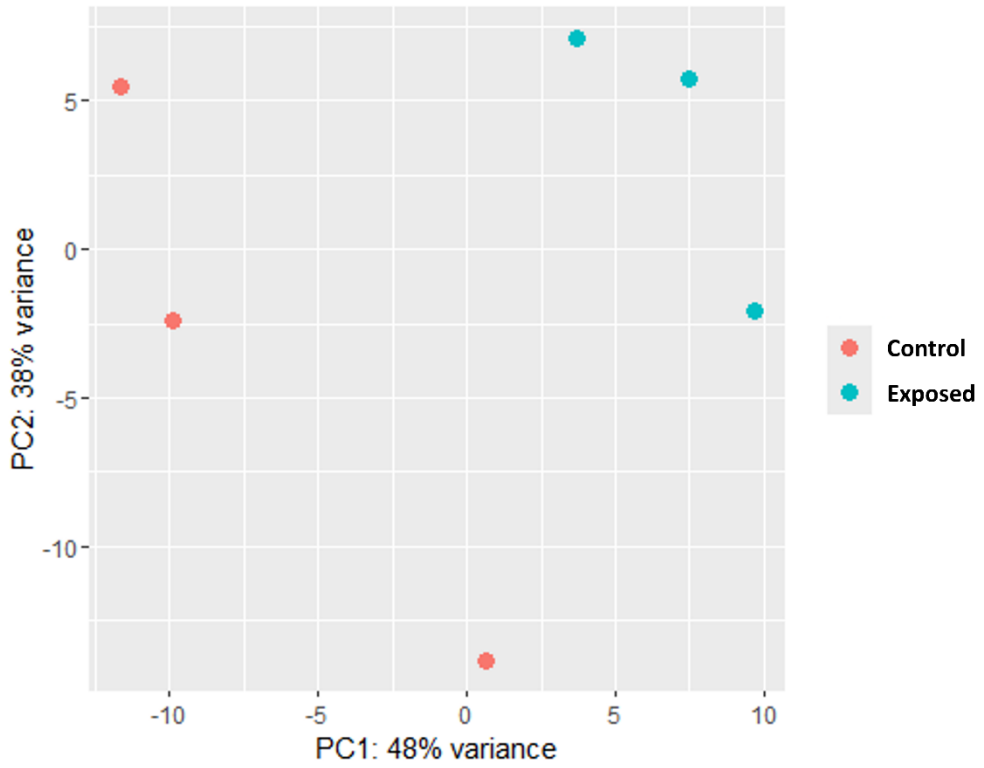


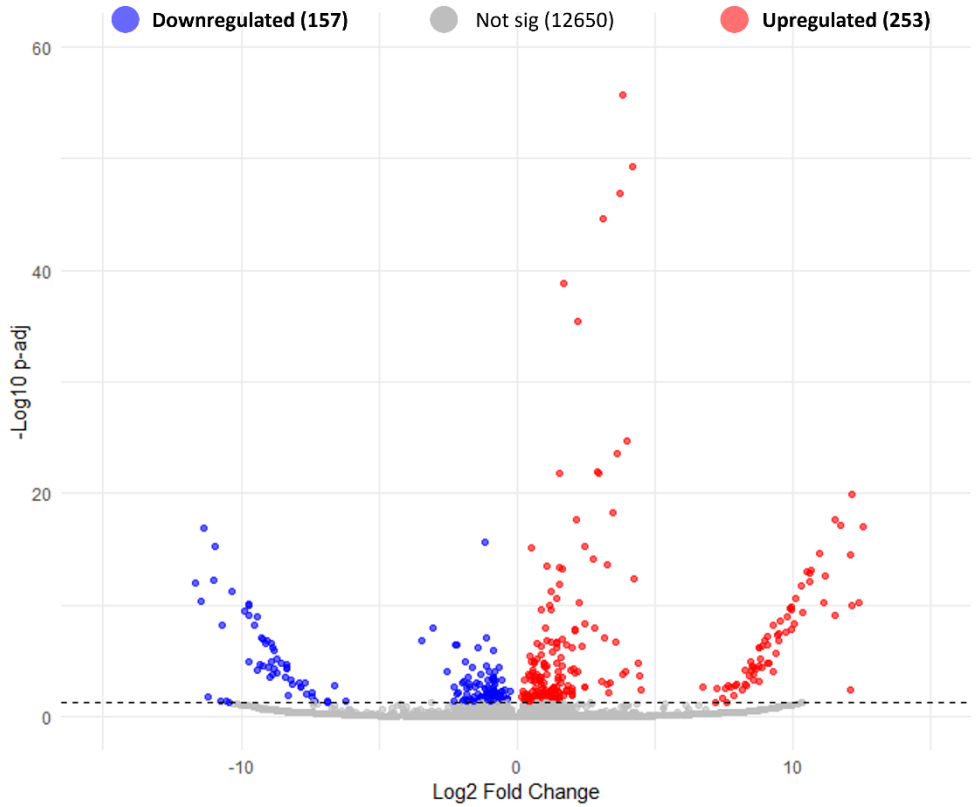


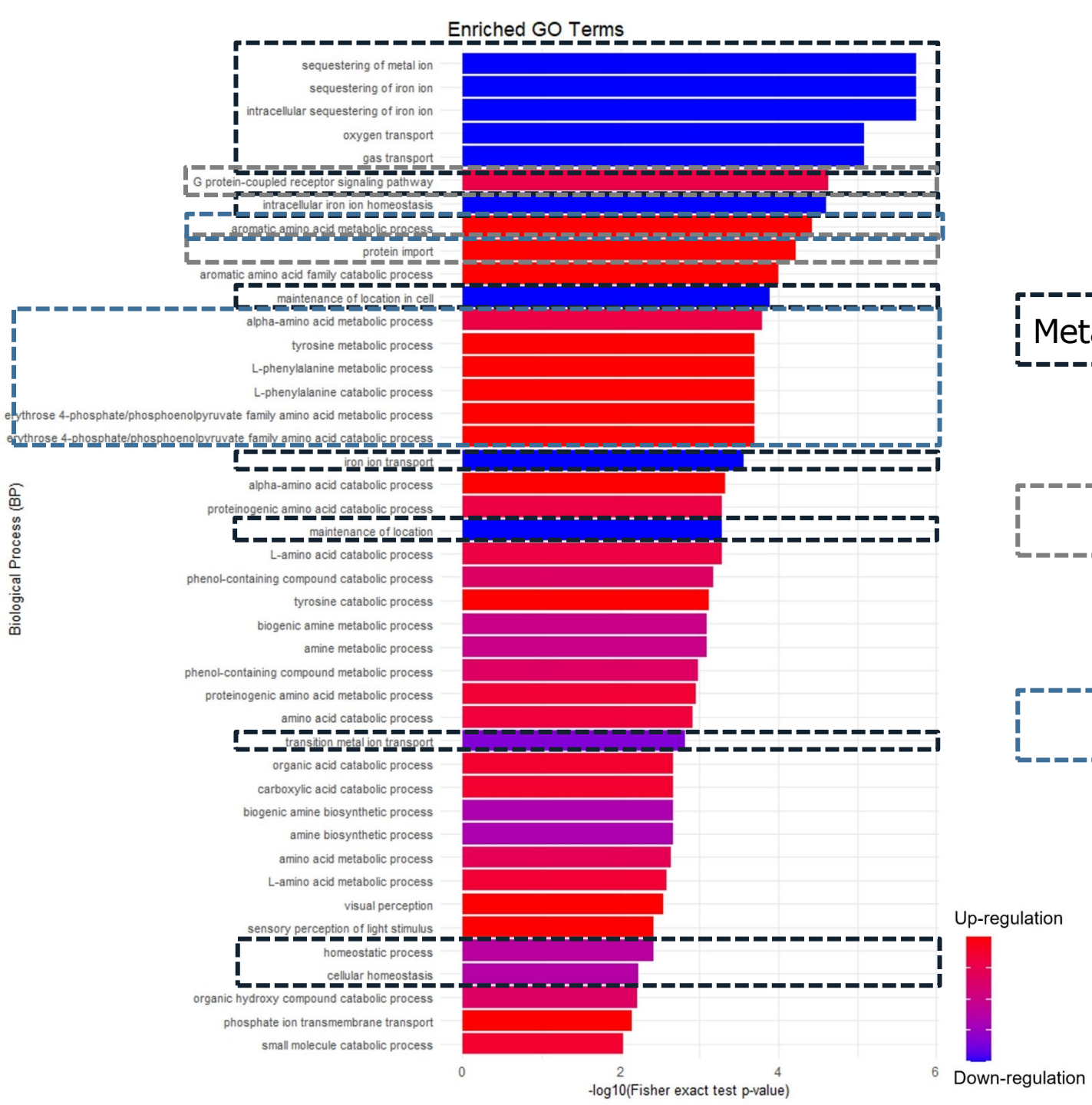
Results & Discussion

Molecular level - Transcriptome

➢ Cd-induced DNA methylation changes probably elicit an altered gene expression profile







Gene expression trend

Metal ion metabolism / Cellular homeostasis ↓

Cell signaling ↑

Amino acids metabolism ↑

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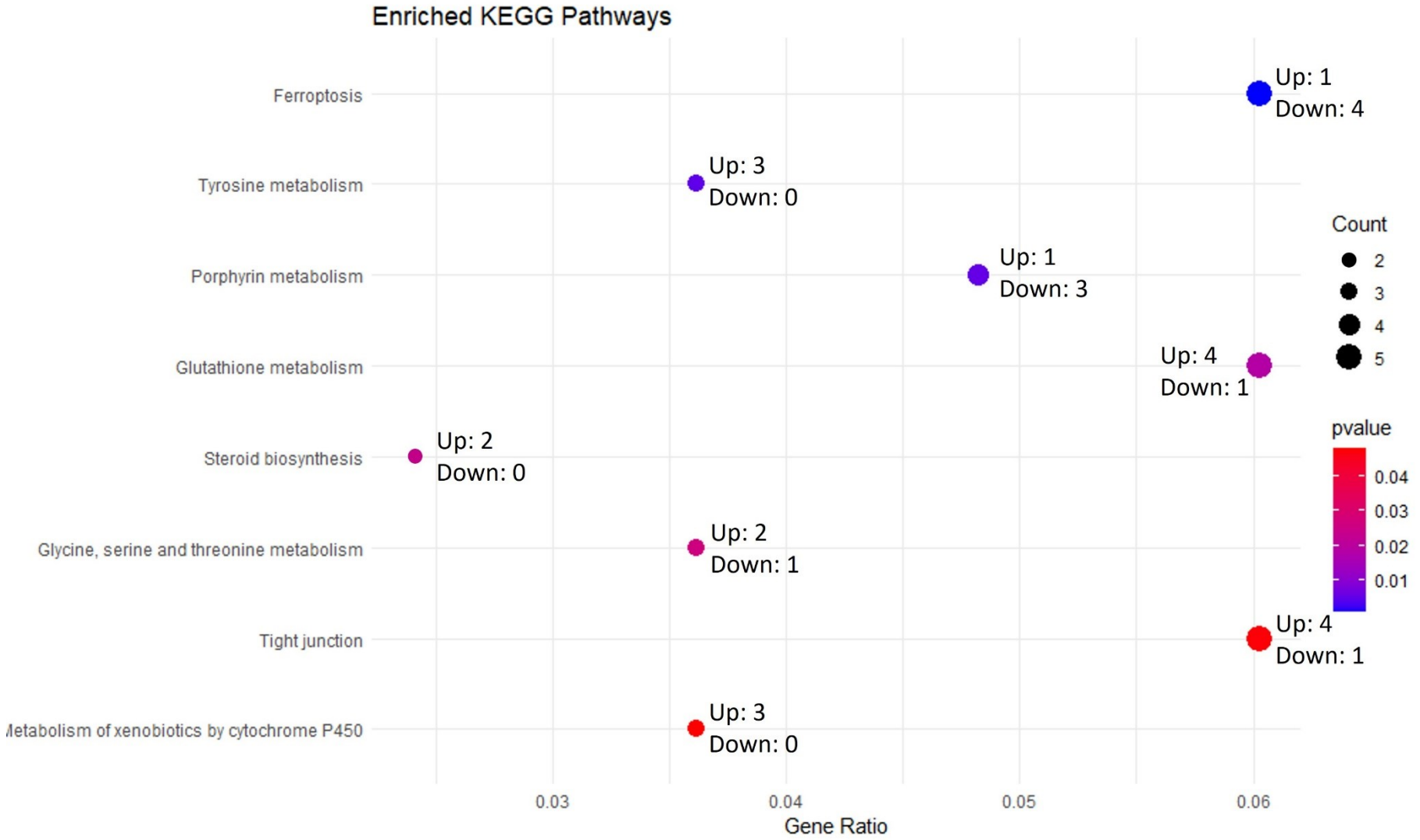

Enriched KEGG Pathways
Ferroptosis
Tyrosine metabolism
Porphyrin metabolism
Glutathione metabolism
Steroid biosynthesis
Glycine, serine and threonine metabolism
Tight junction
Metabolism of xenobiotics by cytochrome P450
Up: 1
Down: 4
Up: 3
Down: 0
Up: 1
Down: 3
Up: 4
Down: 1
Up: 2
Down: 0
Up: 2
Down: 1
Up: 4
Down: 1
Up: 3
Down: 0
Count
2
3
4
5
pvalue
0.04
0.03
0.02
0.01
0.03
0.04
0.05
0.06
Gene Ratio

Results & Discussion

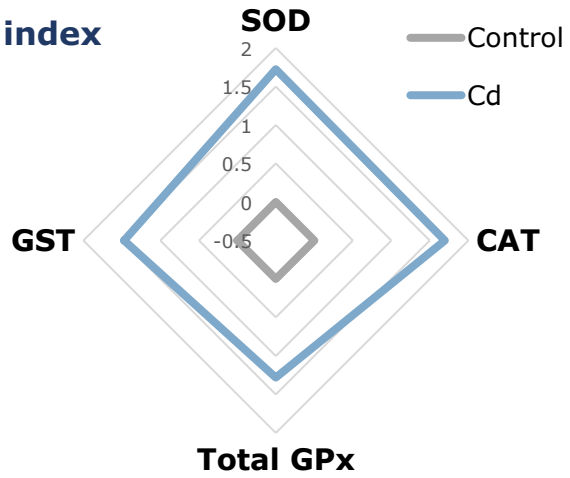
(Sub)cellular level

Transcriptomic response

- Genes involved in the cytochrome P450 and glutathione antioxidant pathways **up-regulated**
- Genes involved in metal metabolism **down-regulated**
- Gene involved in DNA repair **down-regulated**

Antioxidant enzymes activity → **Lipid peroxidation** → Oxidation → **DNA damage**

IBRv2 index



Ferritin heavy chain – LFC = -11.44

DBH-like monooxygenase protein 2 – LFC = -10.71

REV1 – LFC = -7.65

- Cd exposure disrupts other metals metabolism and leads to cell death via ferroptosis (Hong et al., 2022)
- *REV1* loss leads to higher susceptibility to DNA damage (Agyare-Tabbi et al., 2024)

Results & Discussion

Organism level

Transcriptomic response

- Genes involved in calcium homeostasis **down-regulated**
- Genes involved in digestive function and exoskeleton structure **down-regulated**

Heart rate

CLCA4 – LFC = -11.21

Calumenin-A – LFC = -1.71

MEGF6 – LFC = -1.47

Reduced fitness

→

Altered energy allocation

- Detoxification processes
- Reestablishment of lost DNA methylation
- DNA repair processes

Body length

Alpha-amylase – LFC = -11.67

Chymotrypsinogen B-like – LFC = -10.72

KRTAP6-2 – LFC = -10.42

Chitin deacetylase 1 – LFC = -1.30

Populational level effects

- *D. magna* heart rate is regulated by intracellular calcium levels and is a good indicator of daphnids fitness (Pirtle et al., 2018)
- Cd-exposed *D. magna* allocate energy towards detoxification and survival and away from growth and reproduction (Knops et al., 2001)

Main conclusions

The obtained results underpin Cd **sub-lethal exposures** as a significant challenge to *D. magna* thriving

This work provides insights into Cd prolific mechanisms of toxic action considering **DNA methylation as a molecular initiating event**

Acknowledgements

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Thank you for your attention