

Whole Transcriptome Analysis In The Estuarine Amphipod Exposed To Highway Road Dust

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Introduction

- ✓ Urban road dust contains toxic chemicals (e.g., metals, hydrocarbons).
- ✓ Road dust causes **adverse effects on aquatic organisms** after being flushed into receiving waters by rainfall.
- ✓ Causative toxicants in road dust are still unknown.
- ✓ Little is known about the mode of action of urban road dust toxicity to aquatic organisms.



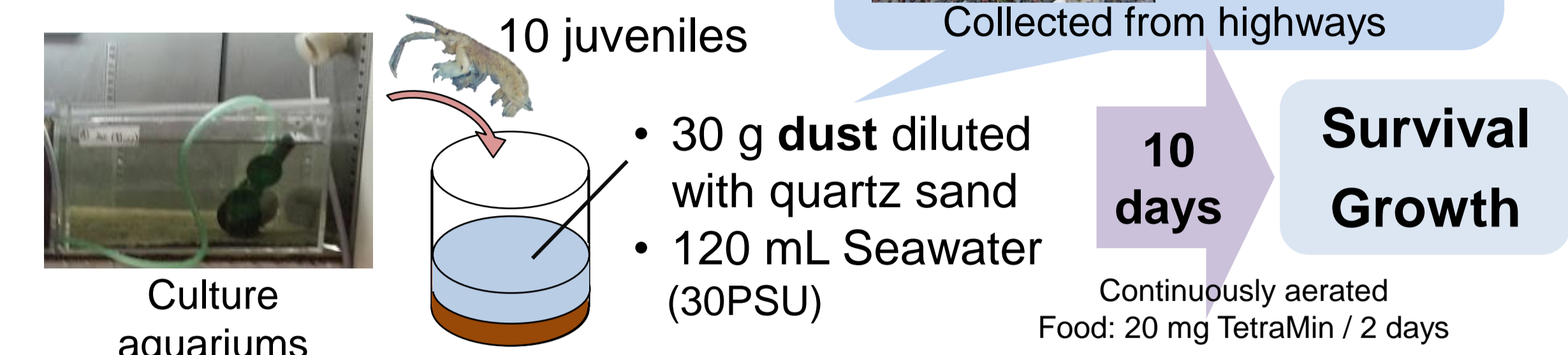
Objective Identify the causative chemicals of road dust toxicity to an estuarine amphipod *Grandidierella japonica*

- using Toxicity Identification Evaluation (TIE)
- using transcriptome analysis

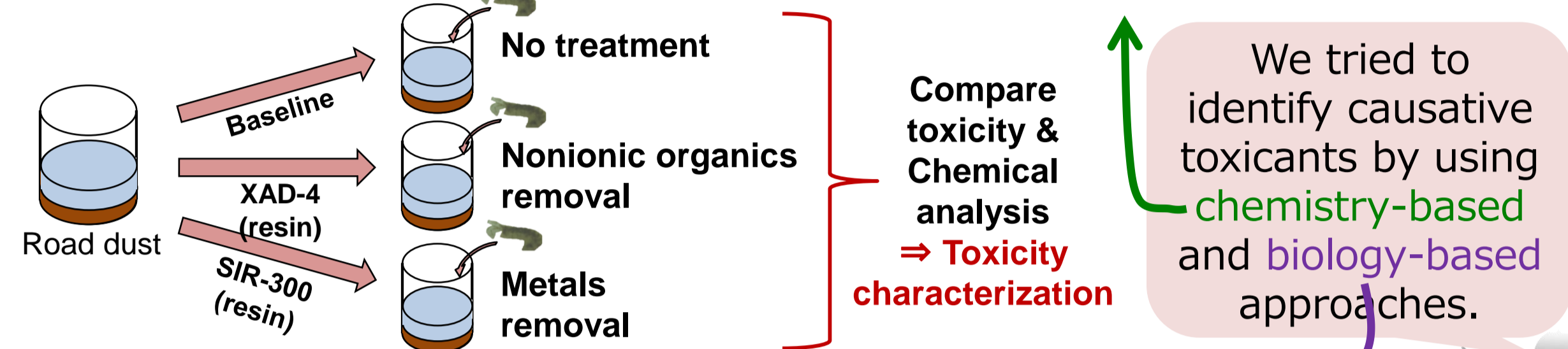


Methods

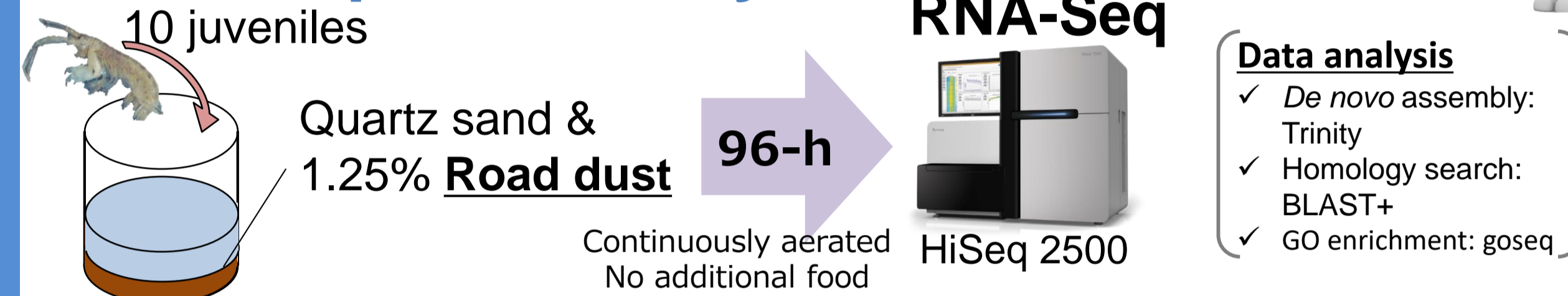
10-day toxicity test [1]



Toxicity Identification Evaluation (TIE) [2]



Transcriptome analysis



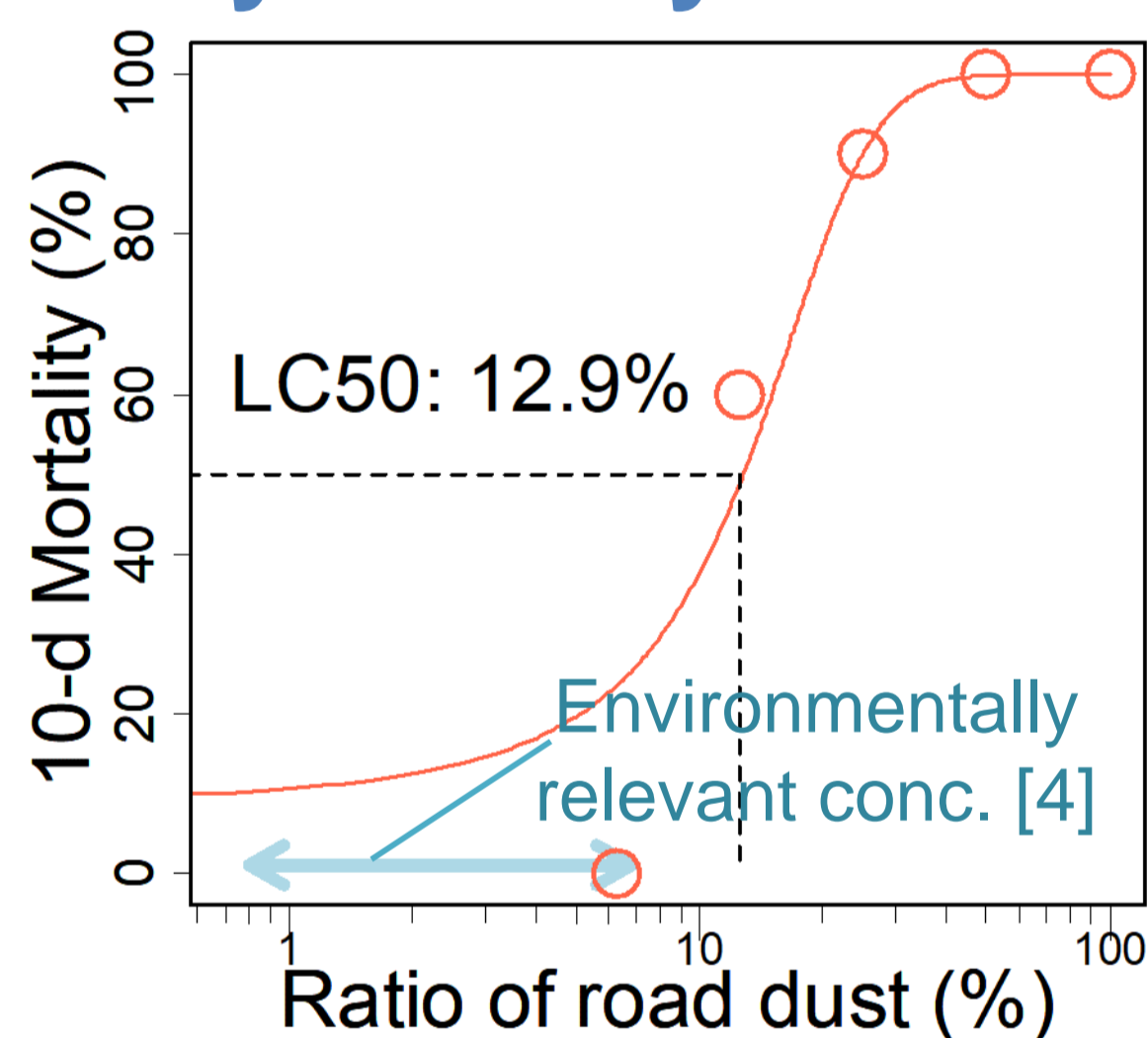
Results & Discussion

Concentrations of toxic chemicals in dust

	Cr	Ni	Cu	Zn	Cd	Pb	Σ12PAHs	Nicotine
Concentration in dust [mg/kg]	163	96	150	1200	0.26	51	<1.09	8.45
Effect Range Median [mg/kg]*	370	52	270	410	9.6	218	-	-

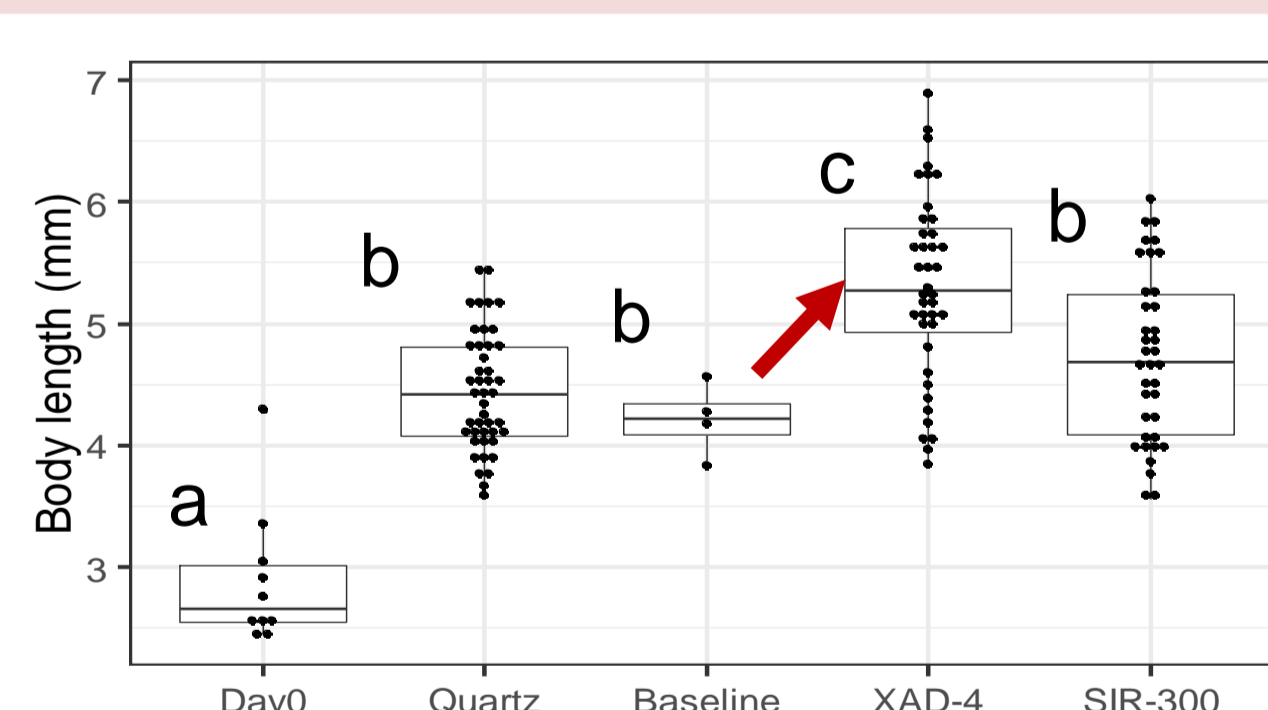
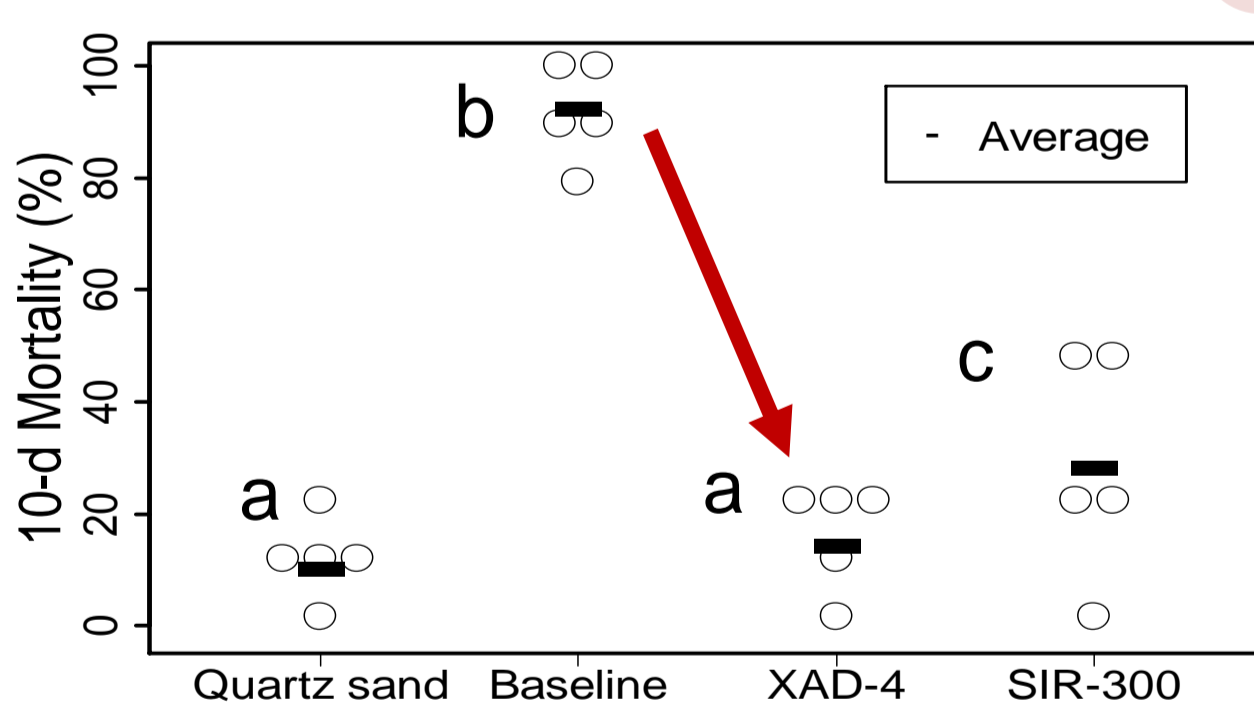
* Effect Range Median (ERM): Major empirical sediment quality guideline [3].

10-day toxicity test & TIE [1]



- ✓ Road dust was lethally toxic to the amphipods.
- ✓ At environmental concentration levels (up to 6.8%), significant toxicity was not observed.

The resin **XAD-4** significantly reduced the road dust toxicity.
⇒ **Organics might be the major contributors to the toxicity.**



Summary of transcriptome analysis

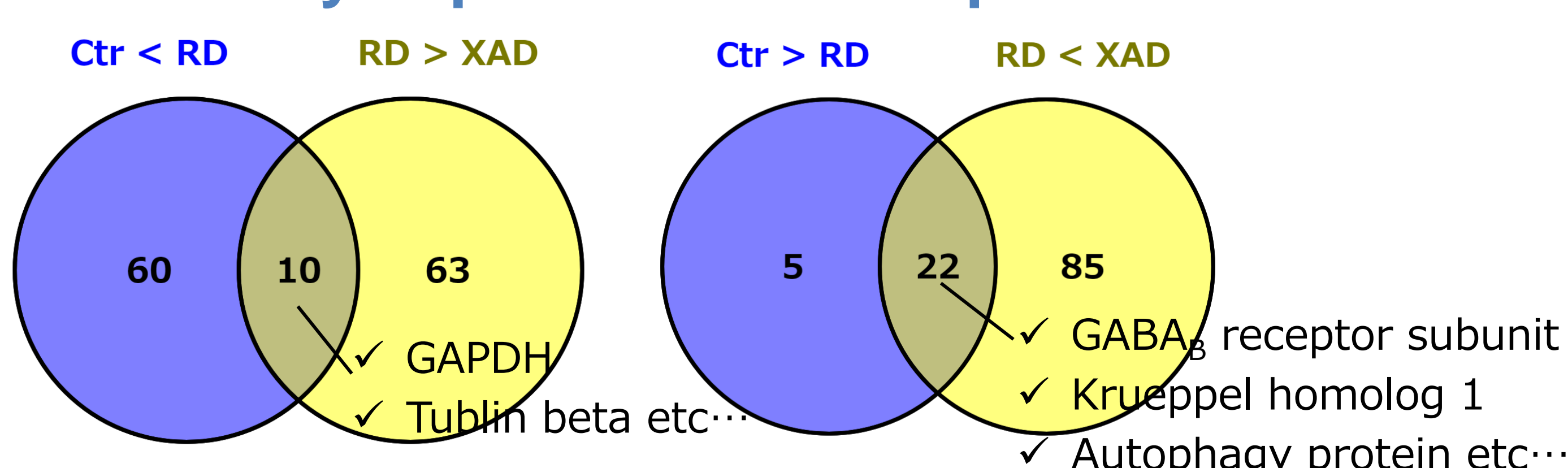
	No. of reads (or base pairs)
Sequenced reads	546,717,510
Total length	(55G bp)
Assembled contigs N50*	259,438 (1,788 bp)
Contigs after removal of low-expressed transcripts	35,912
Annotated reads	16,052

Long enough to be used for ecotoxicological assessments

45% were annotated!

* N50: the sequence length of the shortest contigs at 50% of the total genome length.

Differentially expressed transcripts

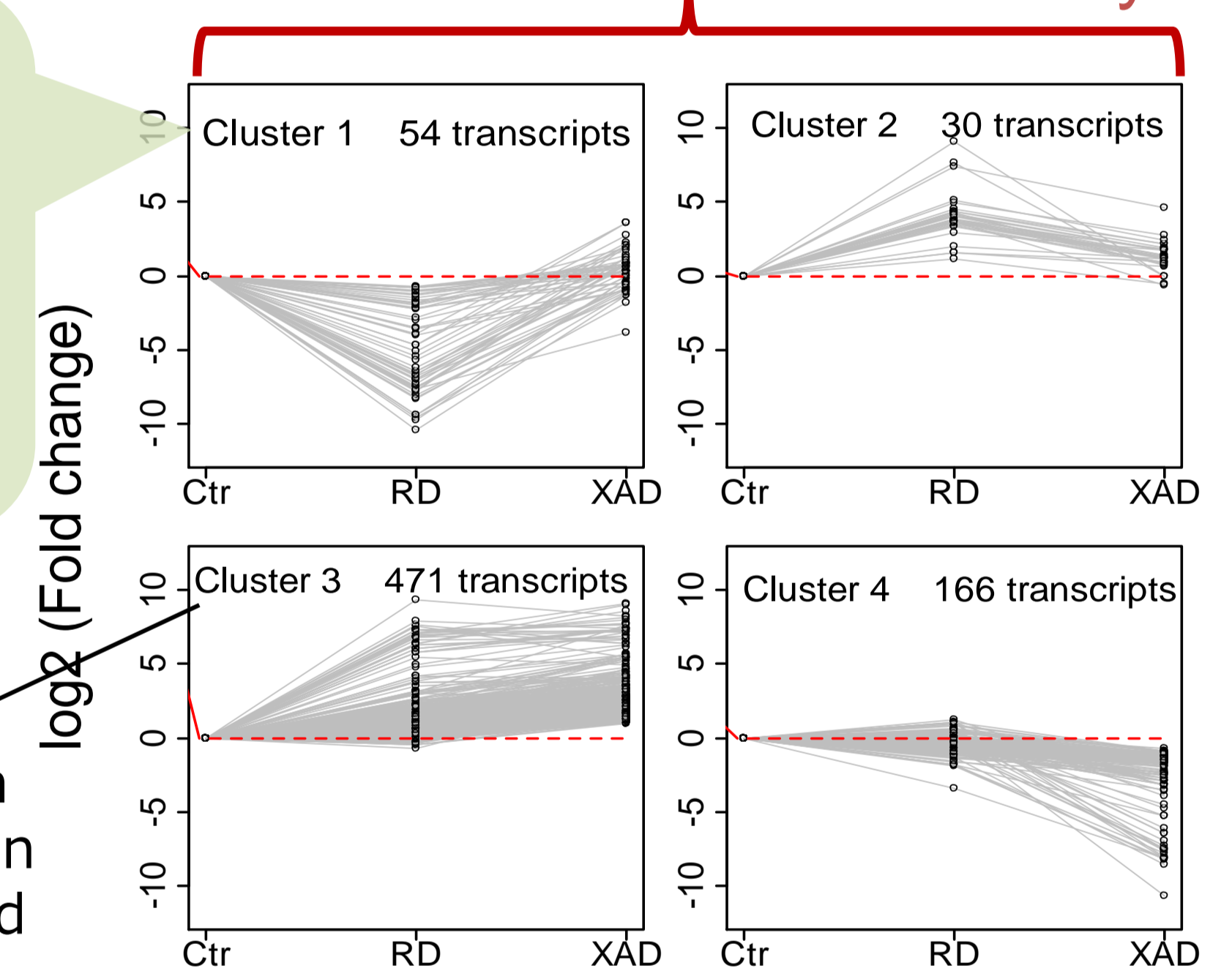


Transcriptome analysis

All 721 differentially expressed transcripts were clustered into 4 groups.

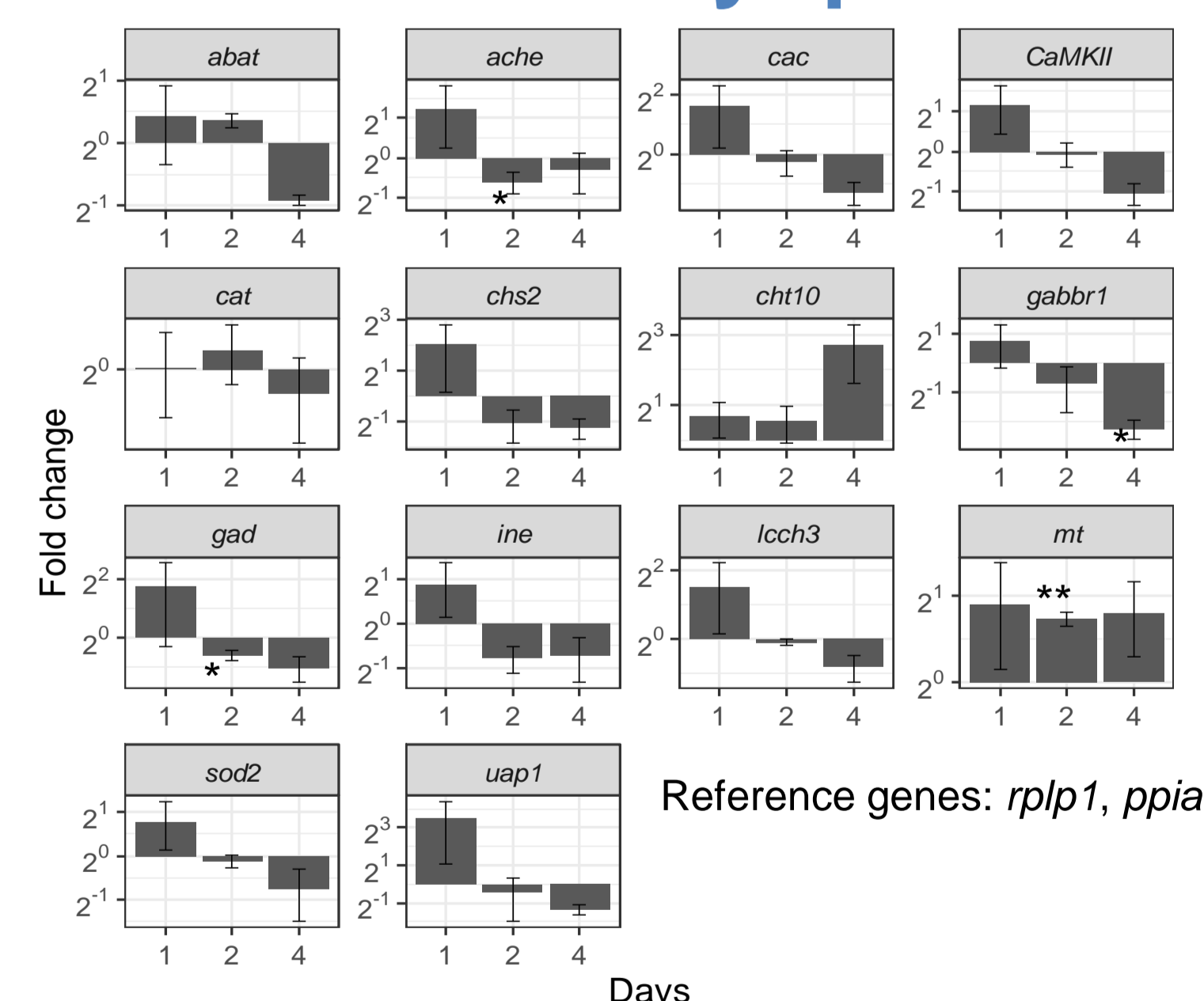
Cluster 1 & 2 should be related to road dust toxicity

Cluster 1
Functions associated with **gamma-aminobutyric acid (GABA) regulation** were enriched.



Functions associated with extracellular region, protein O-linked glycosylation, and chitin catabolic process were enriched in **Cluster 3**.

Confirmation by quantitative PCR



✓ The suppression of GABA_B receptor subunit (*gabbr1*) in the road dust treatment was confirmed by qPCR.

✓ Metallothionein (*mt*) was up-regulated by road dust exposure, possibly due to metals contained in the dust.

Conclusions

- ✓ Sediment TIE indicates that **organics** were the major contributors to the road dust toxicity.
- ✓ Transcriptome analyses revealed that exposure to highway road dust affected gene expression related to **molting and cuticle biosynthesis**. In addition, changes in **GABA signaling pathways** were found in the RD treatment and recovered in XAD treatment.
- ✓ Although the linkage between acute lethality and the transcriptome responses was still unclear, our findings would provide lines of evidence to identify the toxicants in urban road dust.