



Sub-lethal effects in the Western clawed frog exposed to calcium dinonylnaphthalene sulfonate throughout their life-cycle

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Introduction

Naphthalene sulfonic acids under Canada's Chemicals Management Plan (CMP)

- CMP testing 4,300 chemicals from 2006-2020
- Dinonylnaphthalene sulfonic acids are used as an additive in industrial lubricants, greases, corrosion inhibitors, and commercial jet fuels
- Environmental concentrations unknown
 - Log $K_{OW} = 10.96$ (USEPA 2004) so likely in the sediment

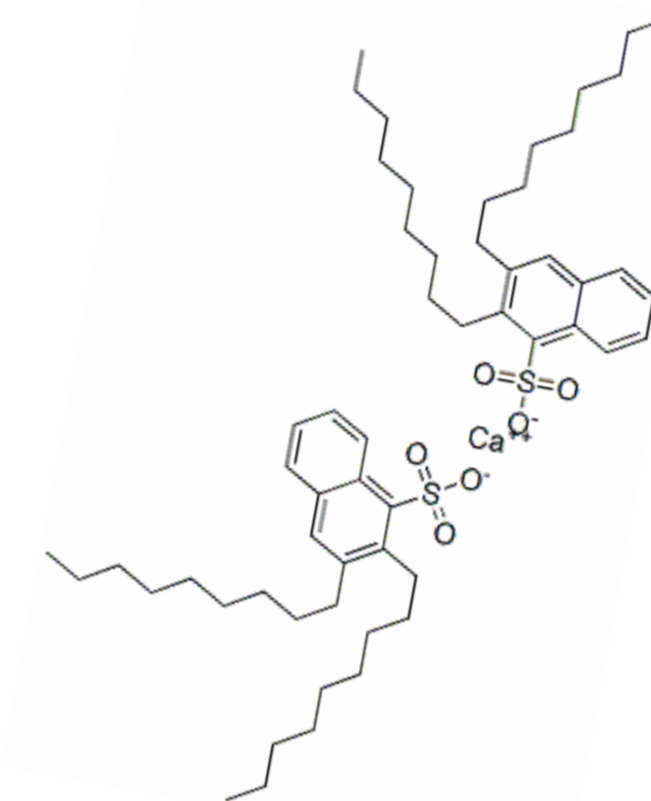


Fig. 1. Chemical structure of CaDNS.

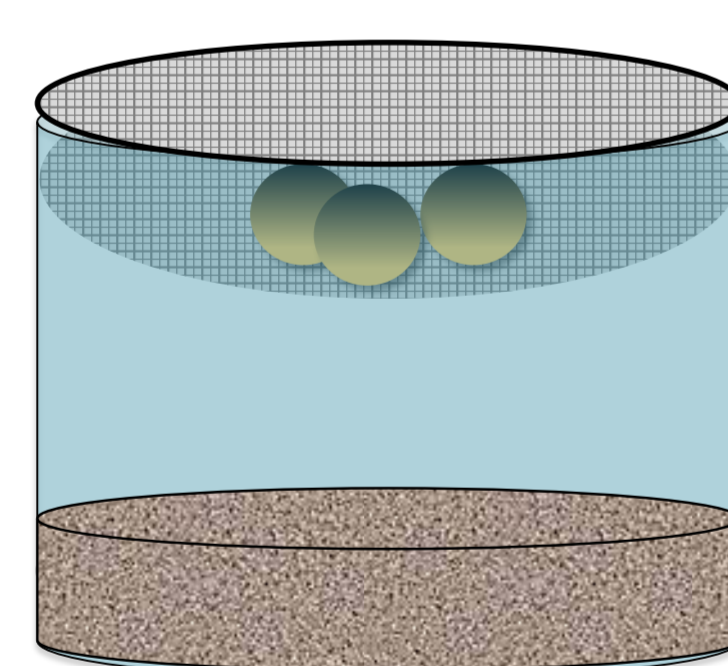
Calcium dinonylnaphthalene sulfonate (CaDNS)

- Limited toxicological information known
 - LD50 > 5000 mg/kg CaDNS in rats (USEPA 2004)
 - 96 h LC50 > 100 mg/L NSA in fish (Greim et al., 1994)

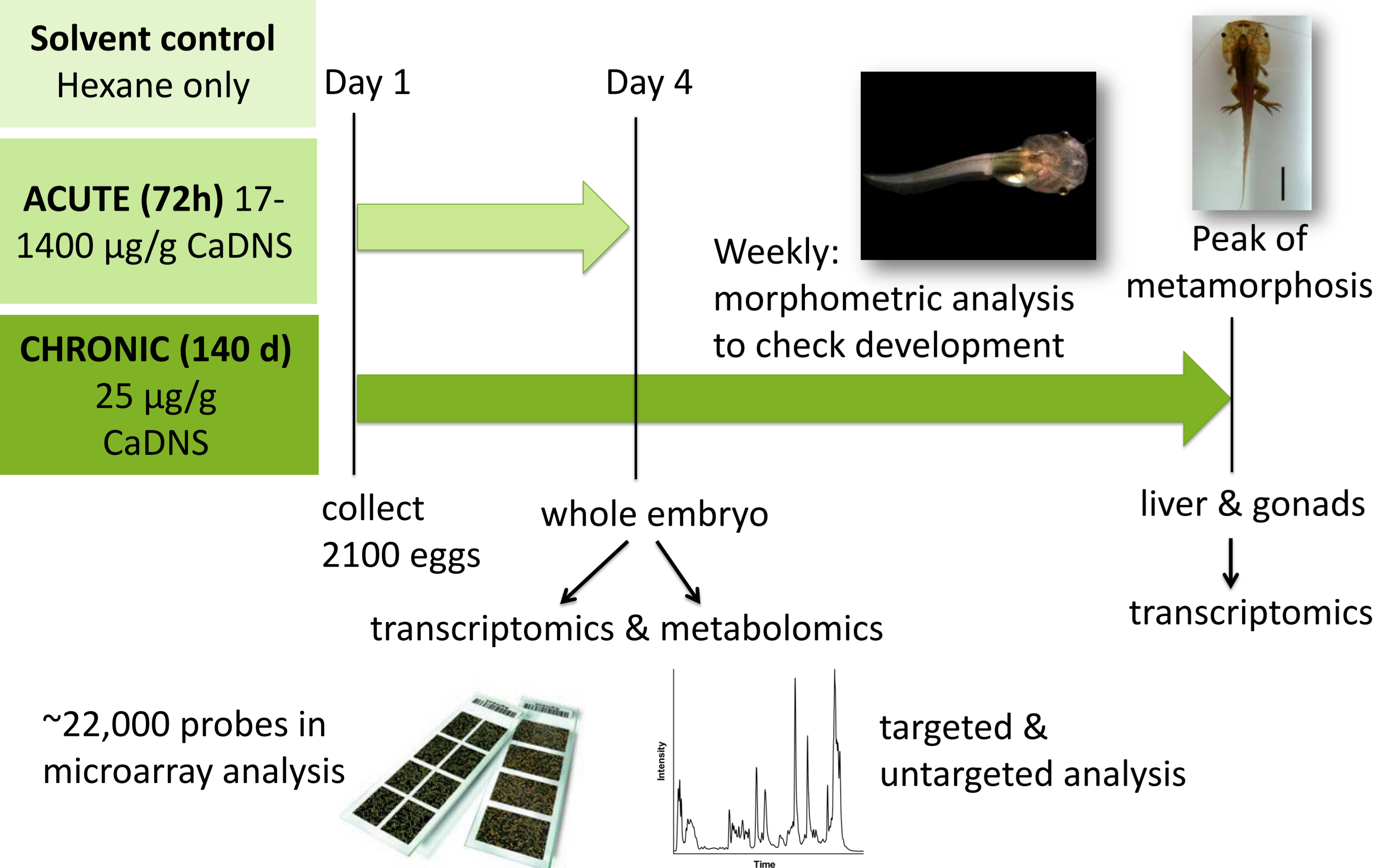
➤ What is the toxicity and sub-lethal effects of CaDNS exposure in the Western clawed frog (*Silurana tropicalis*)?

Methods

Through sediment exposures:



Sand spiked with



Results

Acute exposure (72 h) to CaDNS causes malformations in *S. tropicalis* embryos

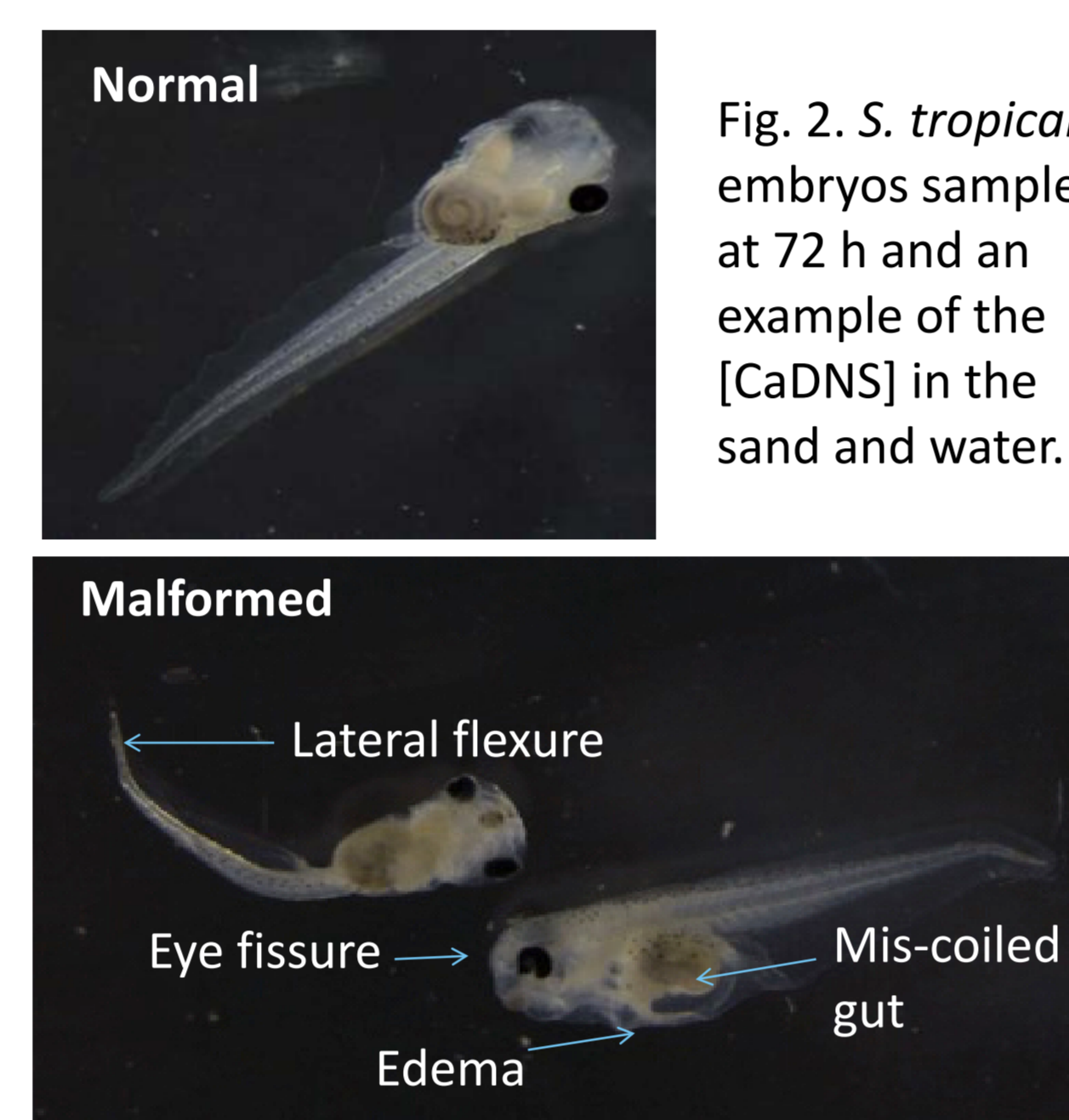


Fig. 2. *S. tropicalis* embryos sampled at 72 h and an example of the [CaDNS] in the sand and water.

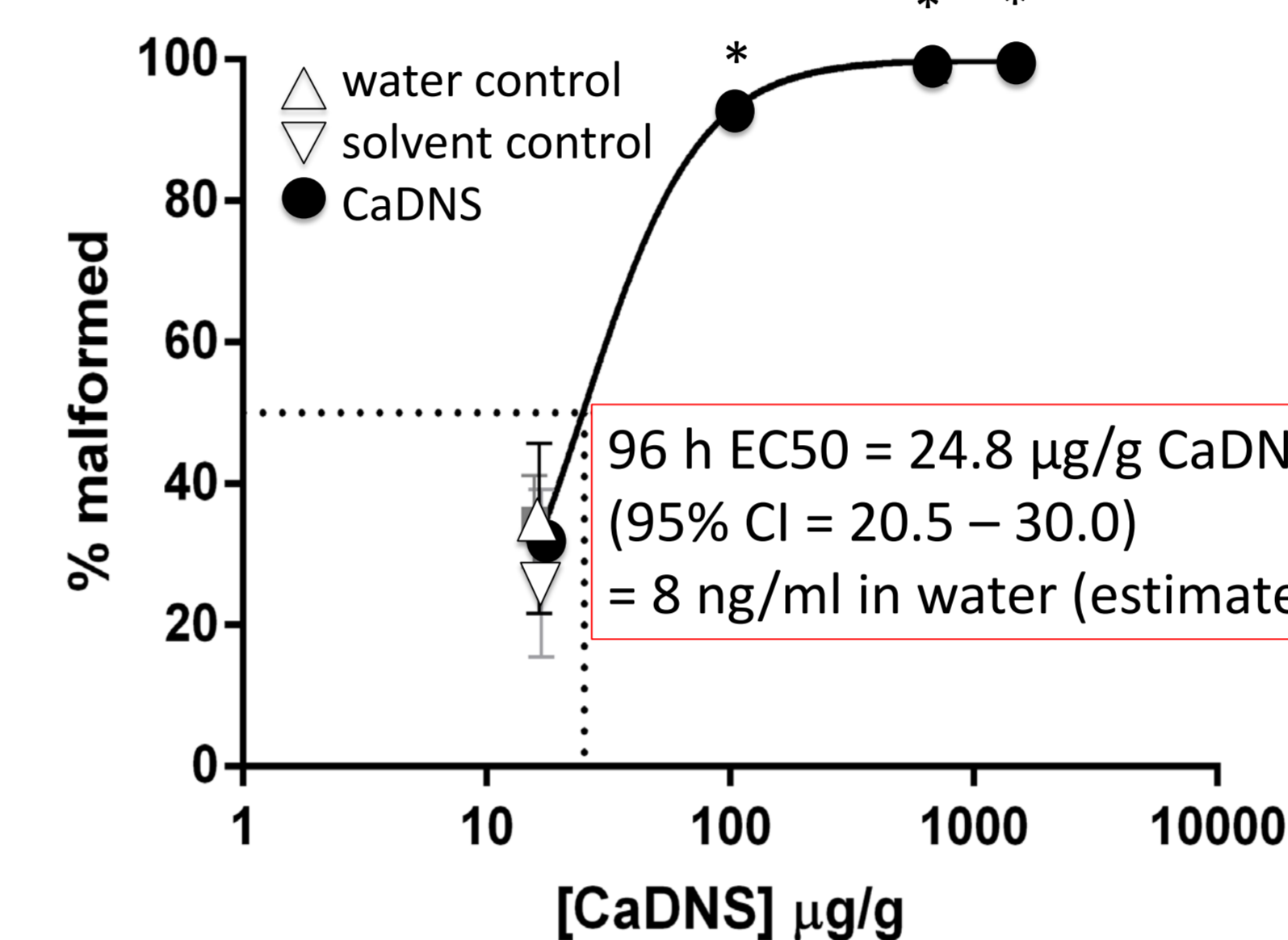


Fig. 3. The percent of embryos that had at least one malformation (% malformed) for a range of [CaDNS] in the sand (on average 90% less in the water).

Over 3,600 differentially expressed genes (DEG) in the transcriptome

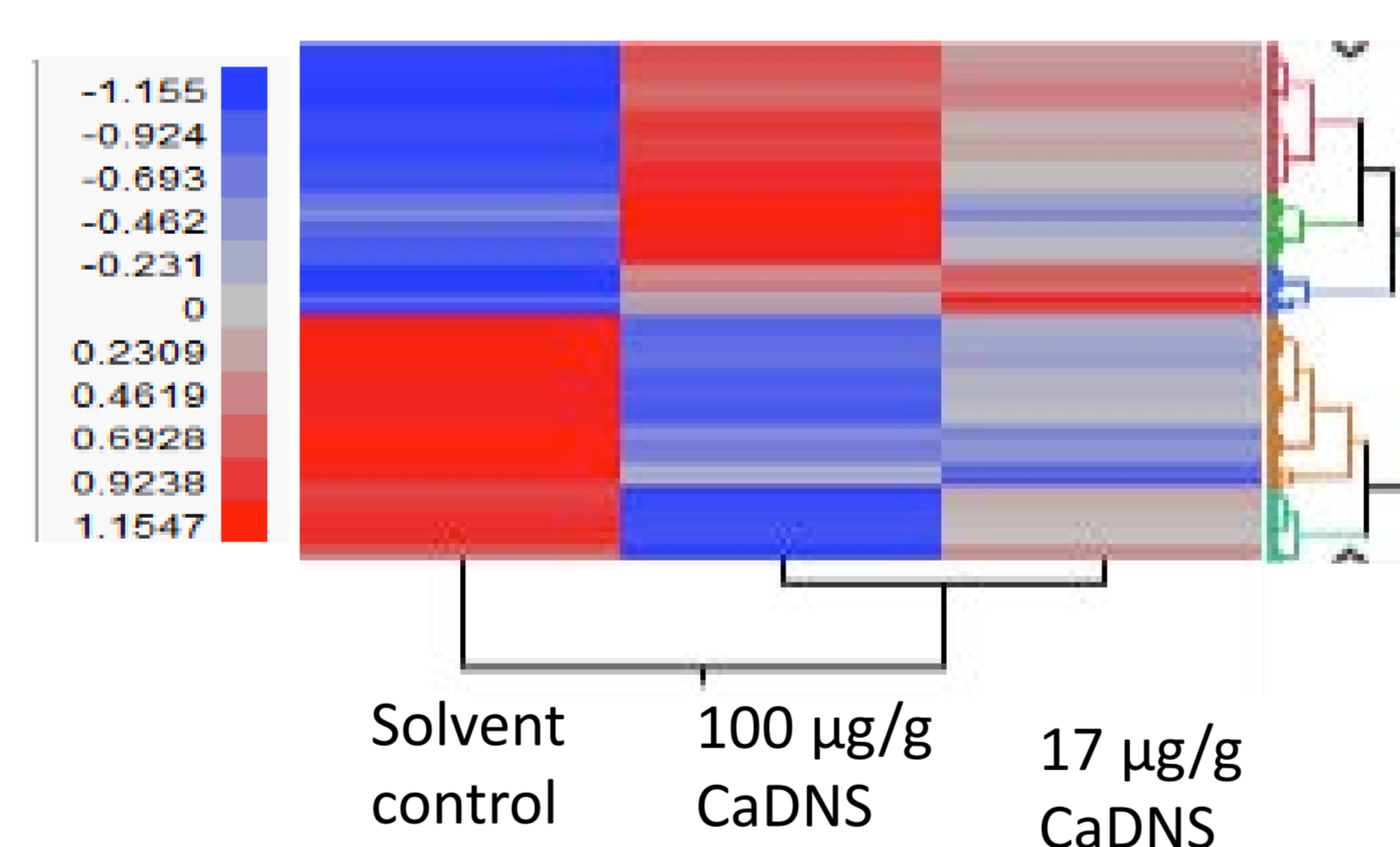


Fig. 4. Supervised heat map of differentially expressed genes (DEG) clustered according to function. Blue represents a down-regulation and red represents an up-regulation.

Table 1. Parametric analysis of gene set enrichment displays the highest number of DEG included in biological processes and molecular functions related to cellular structural development.

Parametric analysis gene set enrichment (PAGE)	# DEGs
actomyosin structure organization	25
cell cycle	6
dendrite development	6
actin binding	25
kinase activity; metal ion binding	7
receptor signaling complex scaffold activity	12

Reduction in metabolite antioxidant capacity

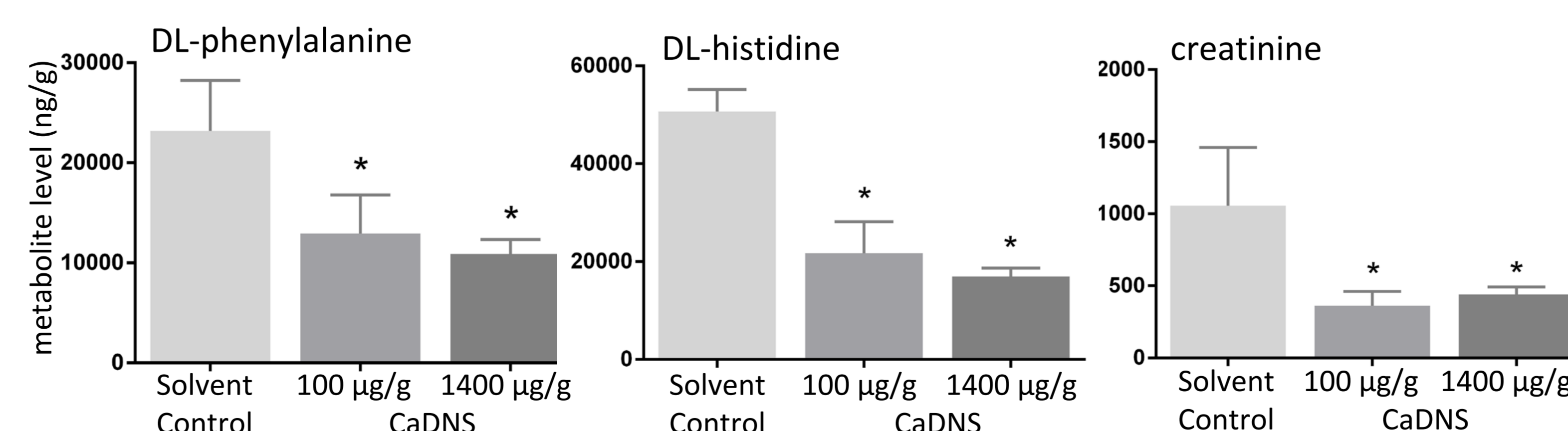


Fig. 5. Metabolite level of essential amino acids (DL-phenylalanine and DL-histidine) with antioxidant function, and creatinine, usually associated with muscle mass and mobility.

Targeted analyses show evidence of oxidative stress

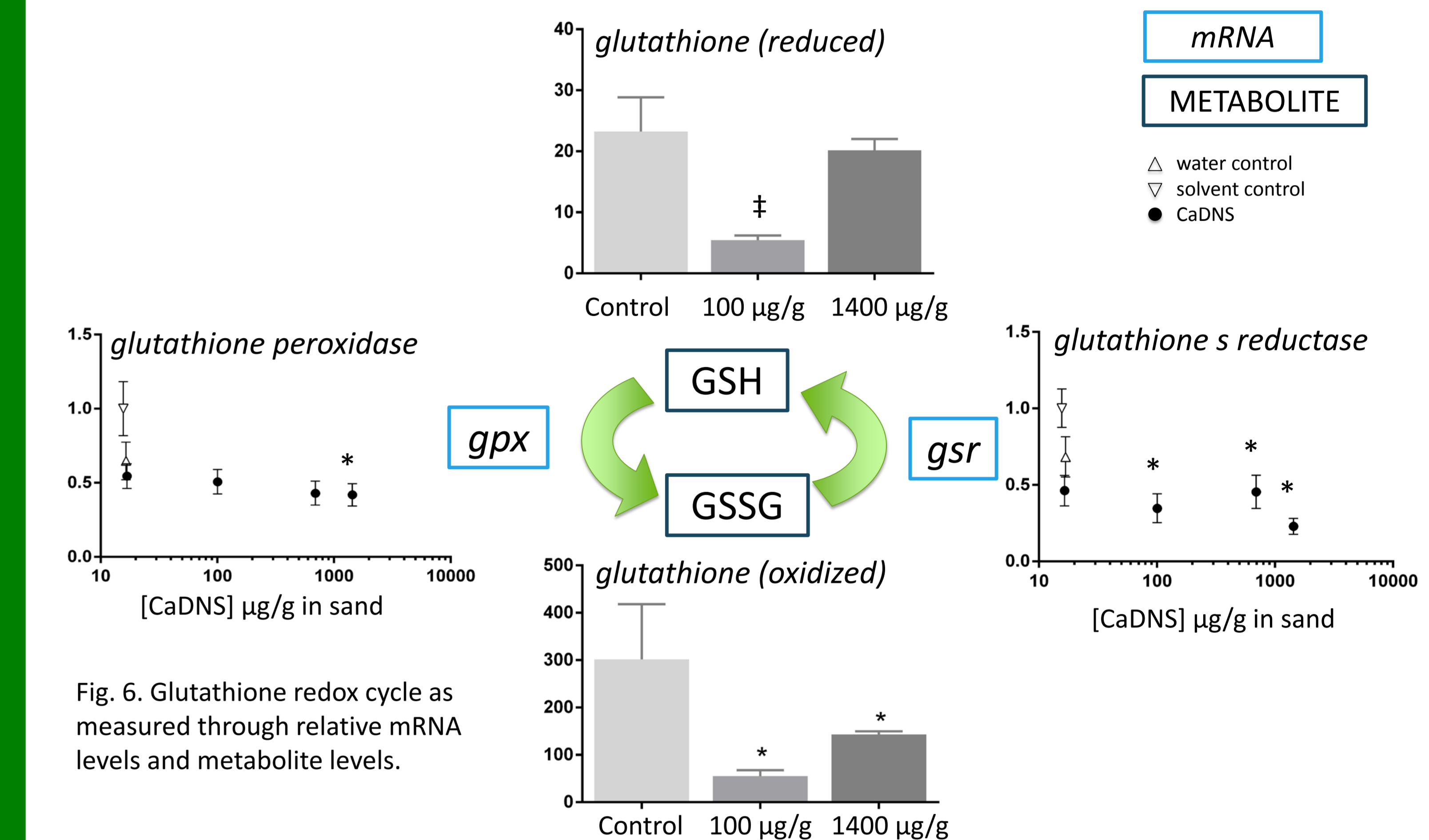


Fig. 6. Glutathione redox cycle as measured through relative mRNA levels and metabolite levels.

Chronic CaDNS exposure causes a developmental delay in metamorphosis

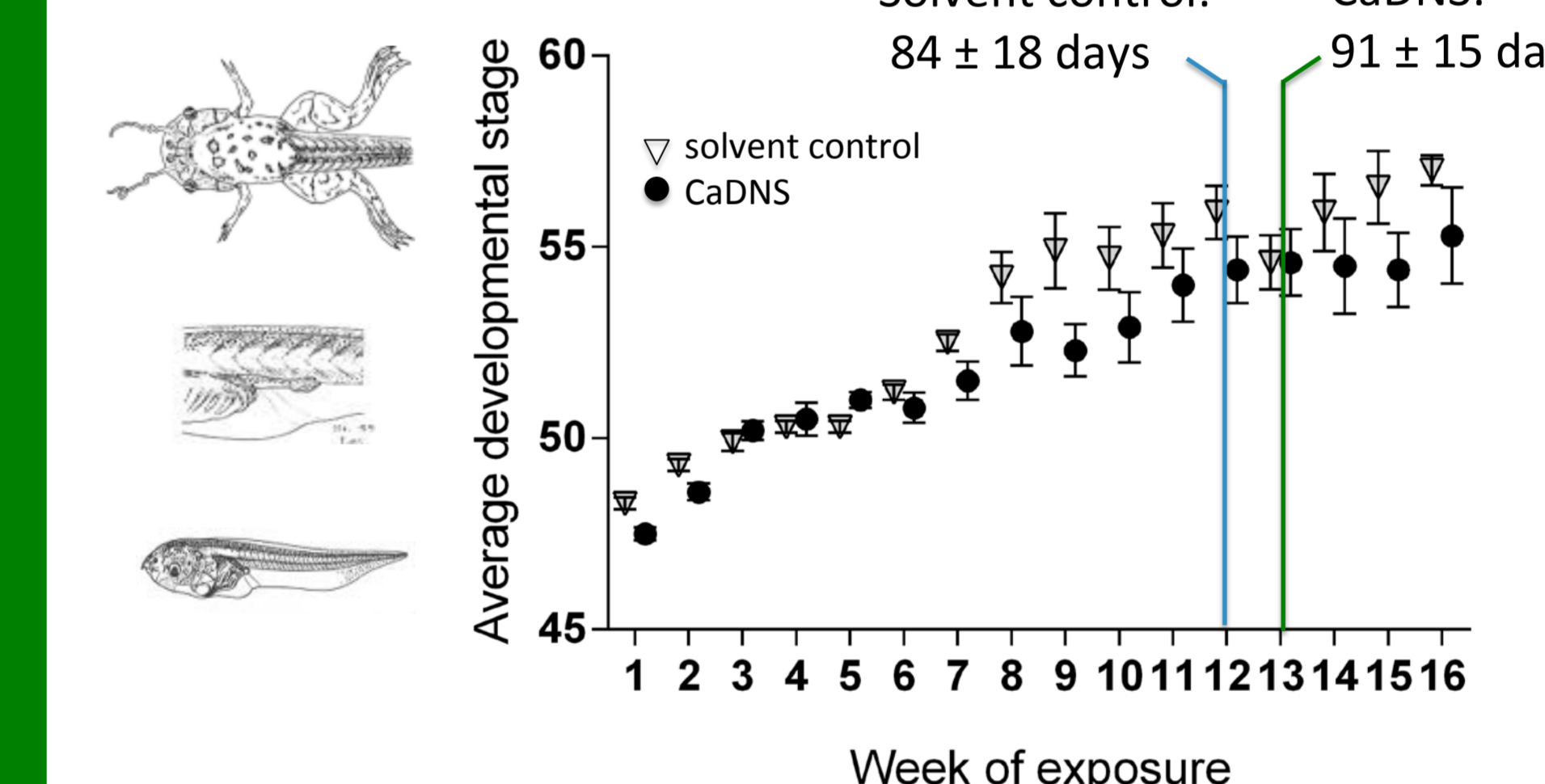


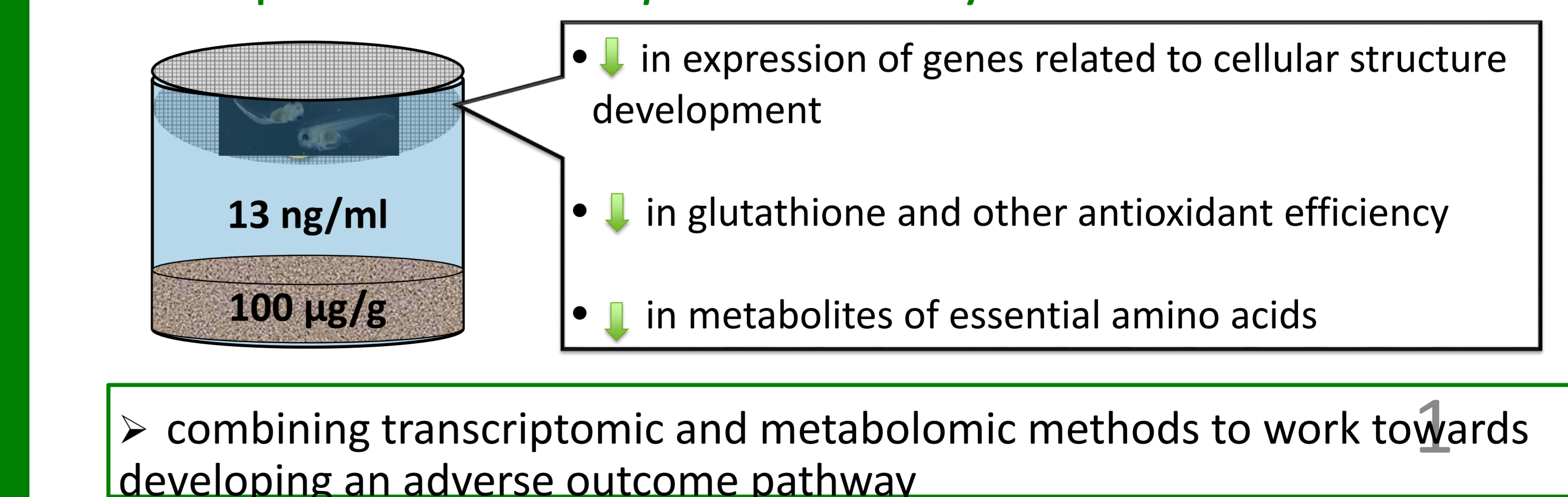
Fig. 7. Weekly average developmental stage (Nieuwkoop and Faber 1994) over the course of the exposure did not differ between solvent control and CaDNS treatment ($p > 0.05$).

When comparing CaDNS exposed embryos to solvent control:

- Increase in average number of days to reach peak of metamorphosis (stage 60) ($p < 0.05$)
- Decrease in total body weight and length at stage 60 ($p < 0.05$)
- No change in genes related to oxidative stress
- Decrease in *dio3* expression (thyroid hormone inactivation)

Preliminary Conclusions

Exposure to low concentration of CaDNS affects development of *S. tropicalis* embryos



Acknowledgements

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