

SUPPLEMENTARY MATERIAL

***Daphnia magna* multigeneration exposure to carbendazim: gene transcription responses**

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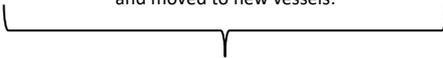
<sup>e</sup>Keygene N.V., Agro Business Park 90, 6708 PW Wageningen, The Netherlands

## Daphnids in clean medium (Control)

1) Multigenerational experiment (F0 to F12): 1 L glass vessels, 3 replicates, 20 *Daphnia magna* each.



At F0 and F12, *D. magna* neonates (<24h) were randomly collected from these 3 vessels and moved to new vessels:



2) Gene transcription analysis (F0 and F12): 150 mL glass vessels, 3 replicates, 5 *D. magna* each.



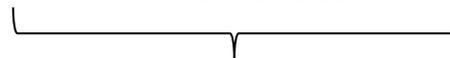
After 10 days, the 5 daphnids were pooled together per replicate - shock-frozen in liquid nitrogen and stored at -80°C until RNA extraction.

## Daphnids in carbendazim ( $5 \mu\text{g L}^{-1}$ )

1) Multigenerational experiment (F0 to F12): 1 L glass vessels, 3 replicates, 20 *Daphnia magna* each.



At F0 and F12, *D. magna* neonates (<24h) were randomly collected from these 3 vessels and moved to new vessels:

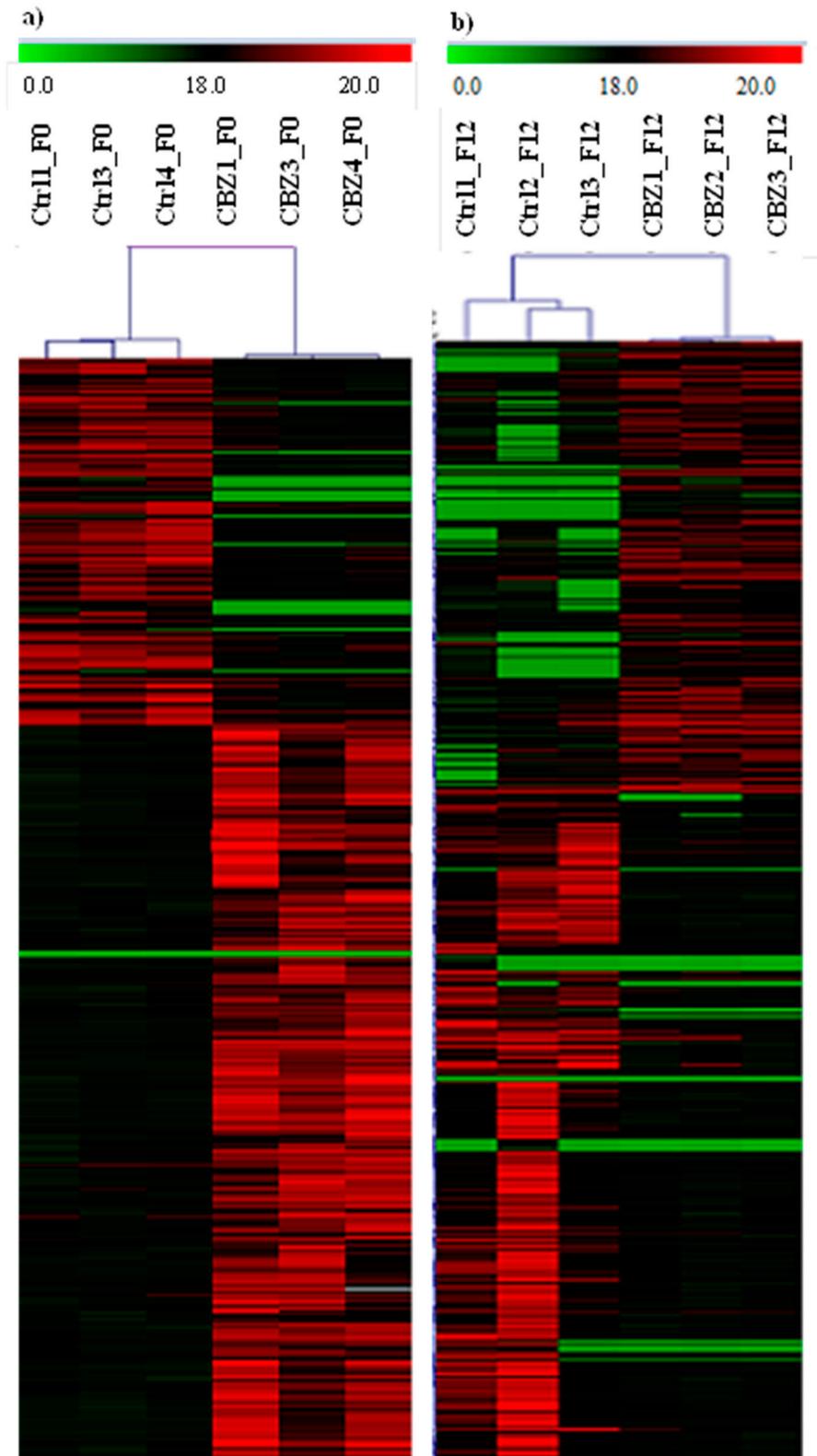


2) Gene transcription analysis (F0 and F12): 150 mL glass vessels, 3 replicates, 5 *D. magna* each.



After 10 days, the 5 daphnids were pooled together per replicate - shock-frozen in liquid nitrogen and stored at -80°C until RNA extraction.

**Figure S1.** Details of the experimental design of the 1) multigenerational experiment and 2) gene transcription analysis. F represents generation. Note: when moved to new vessels, daphnids were maintained in the same conditions (clean daphnids in clean medium; carbendazim daphnids in carbendazim).



**Figure S2.** Hierarchical clustering of statistically significantly expressed genes of *Daphnia magna* exposed to  $5 \mu\text{g L}^{-1}$  carbendazim (CBZ) compared with clean medium (controls, Ctrl): **a)** F0 generation, and **b)** F12 generation. A clear separation and clustering between clean medium conditions and carbendazim exposure is observed, with green shading indicating less expressed genes and red shading indicating highly expressed genes.