

# Supplementary material for

## **Bi-directional effects of vitamin B<sub>12</sub> and methotrexate on *Daphnia magna* fitness and genomic methylation**

Running title: **Invertebrate model for environmental epigenetics**

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**Supplementary Table 1** Summary statistics of generalized linear model testing the effects of vitamin B<sub>12</sub> treatments on fitness of *Daphnia magna*

	Value	Standard Error	Degrees of Freedom	t-value	p-value
Intercept	0.310	0.00048	75	0.004	≤ 0.001
Treatment (B <sub>12</sub> )	0.039	0.0027	75	14.45	≤ 0.001

Generalized linear model (glm) was used to study the relationship between fitness and vitamin B<sub>12</sub> exposure. For every 1 mg/L media increase of vitamin B<sub>12</sub>, the fitness of *D.magna* increased by 0.039. The results revealed by glm are statistically significant ( $P \leq 0.001$ ).

**Supplementary Table 2** Summary statistics of the impact of vitamin B<sub>12</sub> on brood size

	Estimate	Std Error	t value	Pr(> t )
Intercept	33.12	1.65	20.11	<<0.001
Treatment (B <sub>12</sub> )	17.91	0.95	18.93	<<0.001

For every mg/L of vitamin B<sub>12</sub> added to the media, the number of offspring increased by 17 compared to a control lacking vitamin B<sub>12</sub> in media.

**Supplementary Table 3** Summary statistics of generalized linear model testing the effects of MTX exposure on *Daphnia magna* fitness

	Value	Standard Error	Degrees of Freedom	t-value	p-value
Intercept	0.38	0.00059	75	0.0059	≤ 0.001
Treatment	-0.002	0.0002	75	-9.58	≤ 0.001

(Methotrexate)

MTX treatment significantly reduced fitness in *D.magna* by 0.002 for every 1 nM increase of MTX in the media ( $P \leq 0.001$ ).

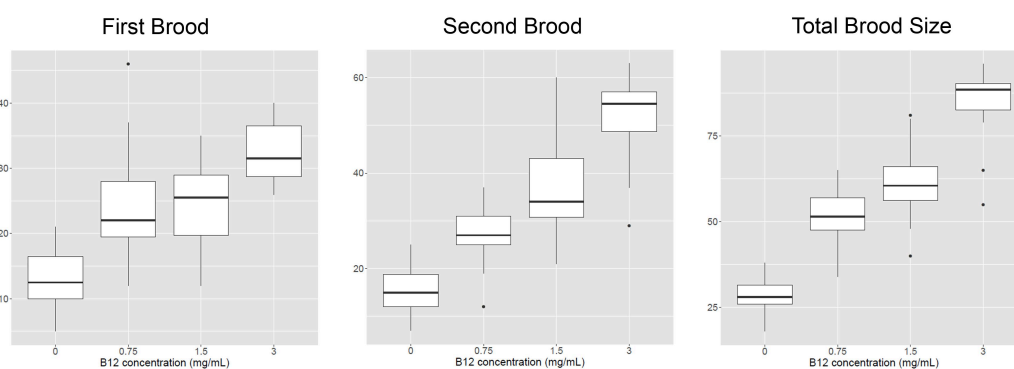
**Supplementary Table 4** Summary statistics of the impact of MTX on brood size

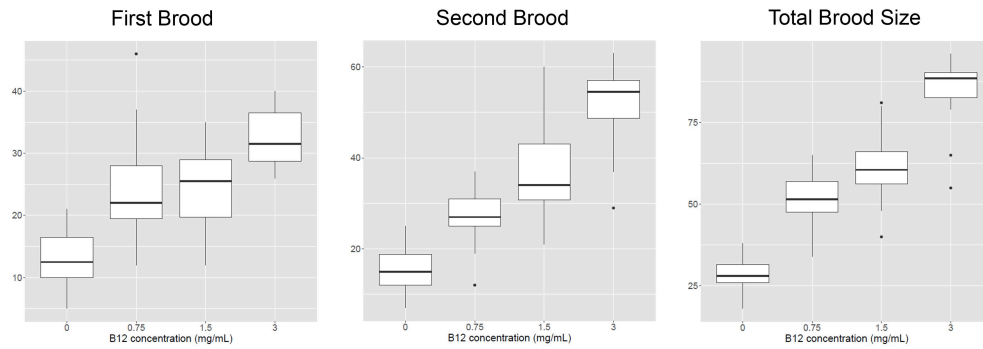
	Estimate	Std Error	t value	Pr(> t )
Intercept	72.0852	3.08	23.43	<< 0.001
Treatment (MTX)	-0.8985	0.11	-8.02	<<0.001

MTX treatment significantly reduced brood size (first and second brood combined) of all exposed *D.magna* mothers. For every 10 nM increase of MTX in the media, the number of offspring decreased by 8.9 compared to the control.

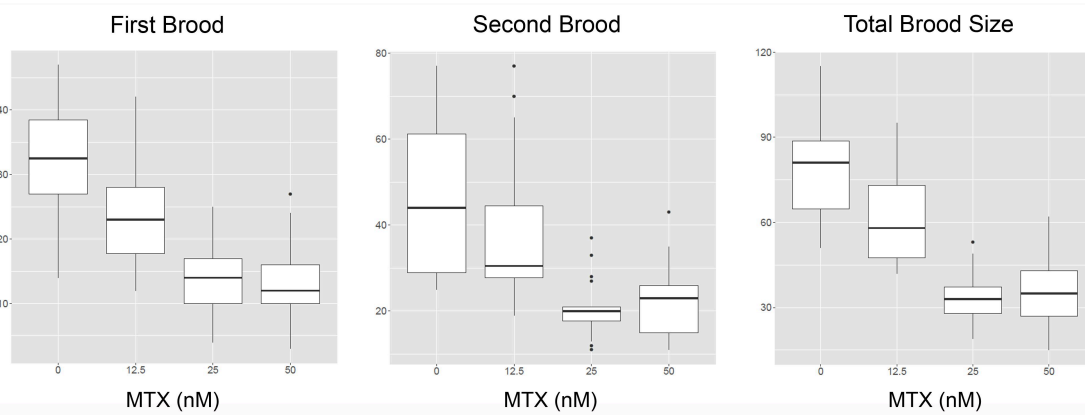
Supplementary figure 1

Impact of B12 exposure on *Daphnia magna* on first, second and total brood size.





Supplementary Figure 2 Impact of MTX exposure on *Daphnia magna* on first, second and total brood size.



Supplementary Figure 3 The global methylation status of mammalian skin cells ( $48.7 \pm 1.2 \%$ ) surpass that of non-mammalian water fleas (Table 5, main text). Amongst *Daphnia*, animals supplemented with vitamin B12 display the highest level of CpG methylation ( $9.09 \pm 2.4 \%$ ) compared to MTX-treated animals and untreated controls (Table 5, main text). A decreasing trend in global methylation levels is observed in *Daphnia* lacking vitamin B12 and exposed to MTX.

### Methylation Sensitive COMET Assay

