**Supplementary material**

**Multi-generation effects of lead (Pb) on two *Daphnia* species: looking at different levels of biological organization**

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Table S1: Two-way ANOVA results testing for effects of treatments (Control, Pb exposure and recovery period) and among generations (F0 to F9) of both Daphnia magna and Daphnia similis and their interaction regarding the acetylcholinesterase activity (AChE). Indicating the sum-of-squares (SS), degrees of freedom (DF), mean squares (MS), the F ratio (F) and the P value (α<0.05).

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| --- | --- | --- | --- | --- | --- |
| Acetylcholinesterase (AChE) activity | | | | | |
| *Dahnia magna* (3x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 75.97 | 6 | 12.66 | F (6, 108) = 30.76 | P < 0.0001 |
| Treatments | 26.86 | 3 | 8.954 | F (3, 108) = 21.75 | P < 0.0001 |
| Generations | 63.07 | 2 | 31.54 | F (2, 108) = 76.61 | P < 0.0001 |
| Residual | 44.46 | 108 | 0.4117 |  |  |
| *Dahnia magna* (1.5x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 28.65 | 6 | 4.776 | F (6, 108) = 13.15 | P < 0.0001 |
| Treatments | 31.16 | 3 | 10.39 | F (3, 108) = 28.61 | P < 0.0001 |
| Generations | 122 | 2 | 60.99 | F (2, 108) = 168.0 | P < 0.0001 |
| Residual | 39.21 | 108 | 0.3631 |  |  |
| *Dahnia similis* (3x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 24.97 | 6 | 4.162 | F (6, 108) = 8.800 | P < 0.0001 |
| Treatments | 67.79 | 3 | 22.6 | F (3, 108) = 47.78 | P < 0.0001 |
| Generations | 80.51 | 2 | 40.26 | F (2, 108) = 85.13 | P < 0.0001 |
| Residual | 51.07 | 108 | 0.4729 |  |  |
| *Dahnia similis* (1.5x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 6.887 | 6 | 1.148 | F (6, 88) = 1.259 | P = 0.2848 |
| Treatments | 47.91 | 3 | 15.97 | F (3, 88) = 17.51 | P < 0.0001 |
| Generations | 123.2 | 2 | 61.58 | F (2, 88) = 67.53 | P < 0.0001 |
| Residual | 80.25 | 88 | 0.9119 |  |  |

Table S2: Two-way ANOVA results testing for effects of treatments (Control, Pb exposure and recovery period) and among generations (F0 to F9) of both Daphnia magna and Daphnia similis and their interaction regarding the Net Reproductive Rate (R0). Indicating the sum-of-squares (SS), degrees of freedom (DF), mean squares (MS), the F ratio (F) and the P value (α<0.05).

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| --- | --- | --- | --- | --- | --- |
| Net Reproductive Rate (R0) | | | | | |
| *Dahnia magna* (3x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 18504 | 4 | 4626 | F (4, 18) = 52.73 | P < 0.0001 |
| Treatments | 9399 | 2 | 4699 | F (2, 18) = 53.56 | P < 0.0001 |
| Generations | 925.2 | 2 | 462.6 | F (2, 18) = 5.273 | P = 0.0158 |
| Residual | 1579 | 18 | 87.73 |  |  |
| *Dahnia magna* (1.5x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 22363 | 4 | 5591 | F (4, 18) = 247.3 | P < 0.0001 |
| Treatments | 5993 | 2 | 2997 | F (2, 18) = 132.6 | P < 0.0001 |
| Generations | 8996 | 2 | 4498 | F (2, 18) = 199.0 | P < 0.0001 |
| Residual | 406.9 | 18 | 22.6 |  |  |
| *Dahnia similis* (3x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 13635 | 4 | 3409 | F (4, 18) = 101.8 | P < 0.0001 |
| Treatments | 2624 | 2 | 1312 | F (2, 18) = 39.17 | P < 0.0001 |
| Generations | 1351 | 2 | 675.7 | F (2, 18) = 20.17 | P < 0.0001 |
| Residual | 602.9 | 18 | 33.5 |  |  |
| *Dahnia similis* (1.5x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 10701 | 4 | 2675 | F (4, 18) = 60.39 | P < 0.0001 |
| Treatments | 3754 | 2 | 1877 | F (2, 18) = 42.37 | P < 0.0001 |
| Generations | 1492 | 2 | 746.1 | F (2, 18) = 16.84 | P < 0.0001 |
| Residual | 797.4 | 18 | 44.3 |  |  |

Table S3: Two-way ANOVA results testing for effects of treatments (Control, Pb exposure and recovery period) and among generations (F0 to F9) of both Daphnia magna and Daphnia similis and their interaction regarding hatching delay. Indicating the sum-of-squares (SS), degrees of freedom (DF), mean squares (MS), the F ratio (F) and the P value (α<0.05).

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| --- | --- | --- | --- | --- | --- |
| Haching delay | | | | | |
| *Dahnia magna* (3x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 64.39 | 6 | 10.73 | F (6, 24) = 3.018 | P = 0.0242 |
| Treatments | 90.06 | 2 | 45.03 | F (2, 24) = 12.66 | P = 0.0002 |
| Generations | 74.44 | 3 | 24.81 | F (3, 24) = 6.979 | P = 0.0015 |
| Residual | 85.33 | 24 | 3.556 |  |  |
| *Dahnia magna* (1.5x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 127.6 | 6 | 21.26 | F (6, 24) = 3.610 | P = 0.0108 |
| Treatments | 113.6 | 2 | 56.78 | F (2, 24) = 9.642 | P = 0.0008 |
| Generations | 410.5 | 3 | 136.8 | F (3, 24) = 23.24 | P < 0.0001 |
| Residual | 141.3 | 24 | 5.889 |  |  |
| *Dahnia similis* (3x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 97.5 | 6 | 16.25 | F (6, 24) = 3.232 | P = 0.0180 |
| Treatments | 77.39 | 2 | 38.69 | F (2, 24) = 7.696 | P = 0.0026 |
| Generations | 81 | 3 | 27 | F (3, 24) = 5.370 | P = 0.0057 |
| Residual | 120.7 | 24 | 5.028 |  |  |
| *Dahnia similis* (1.5x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 121.5 | 6 | 20.25 | F (6, 24) = 4.028 | P = 0.0062 |
| Treatments | 145.4 | 2 | 72.69 | F (2, 24) = 14.46 | P < 0.0001 |
| Generations | 598.8 | 3 | 199.6 | F (3, 24) = 39.70 | P < 0.0001 |
| Residual | 120.7 | 24 | 5.028 |  |  |

Table S4: Two-way ANOVA results testing for effects of treatments (Control, Pb exposure and recovery period) and among generations (F0 to F9) of both Daphnia magna and Daphnia similis and their interaction regarding lifespan. Indicating the sum-of-squares (SS), degrees of freedom (DF), mean squares (MS), the F ratio (F) and the P value (α<0.05).

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| --- | --- | --- | --- | --- | --- |
| Lifespan | | | | | |
| *Dahnia magna* (3x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 2203 | 4 | 550.8 | F (4, 18) = 34.19 | P < 0.0001 |
| Treatments | 1989 | 2 | 994.3 | F (2, 18) = 61.72 | P < 0.0001 |
| Generations | 712.7 | 2 | 356.3 | F (2, 18) = 22.12 | P < 0.0001 |
| Residual | 290 | 18 | 16.11 |  |  |
| *Dahnia magna* (1.5x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 746 | 4 | 186.5 | F (4, 18) = 10.23 | P = 0.0002 |
| Treatments | 314 | 2 | 157 | F (2, 18) = 8.616 | P = 0.0024 |
| Generations | 1400 | 2 | 700 | F (2, 18) = 38.41 | P < 0.0001 |
| Residual | 328 | 18 | 18.22 |  |  |
| *Dahnia similis* (3x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 1236 | 4 | 309 | F (4, 18) = 7.242 | P = 0.0012 |
| Treatments | 345.4 | 2 | 172.7 | F (2, 18) = 4.048 | P = 0.0353 |
| Generations | 1908 | 2 | 953.8 | F (2, 18) = 22.36 | P < 0.0001 |
| Residual | 768 | 18 | 42.67 |  |  |
| *Dahnia similis* (1.5x105cells/mL) | | | | | |
| ANOVA table | SS | DF | MS | F (DFn, DFd) | P value |
| Interaction | 1529 | 4 | 382.2 | F (4, 18) = 24.22 | P < 0.0001 |
| Treatments | 422.7 | 2 | 211.4 | F (2, 18) = 13.40 | P = 0.0003 |
| Generations | 1973 | 2 | 986.3 | F (2, 18) = 62.51 | P < 0.0001 |
| Residual | 284 | 18 | 15.78 |  |  |