

One like all? Behavioral response range of native and invasive amphipods to neonicotinoid exposure

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Supplementary Material

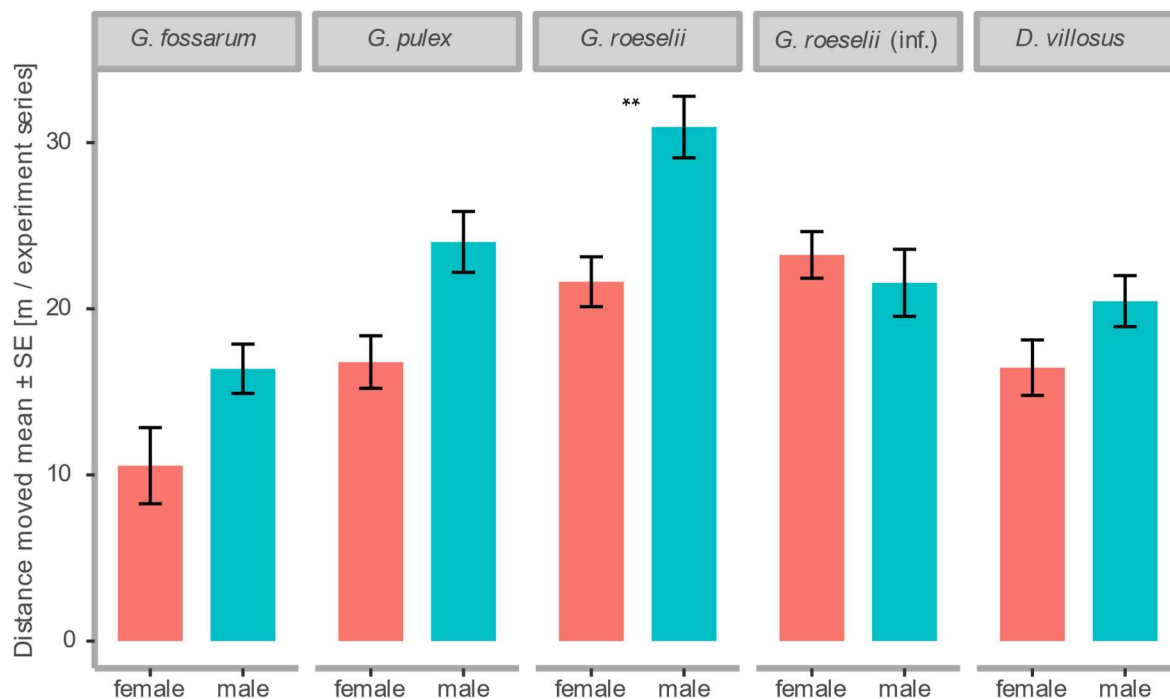


Figure S 1 Sex specific behavior of four amphipod species and infected *G. roeselii* given as total distance moved per experiment. Values are based on estimated marginal means. Error bars show the standard error. Asterisks indicate significant deviations between the sexes, based on Tukey's HSD post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

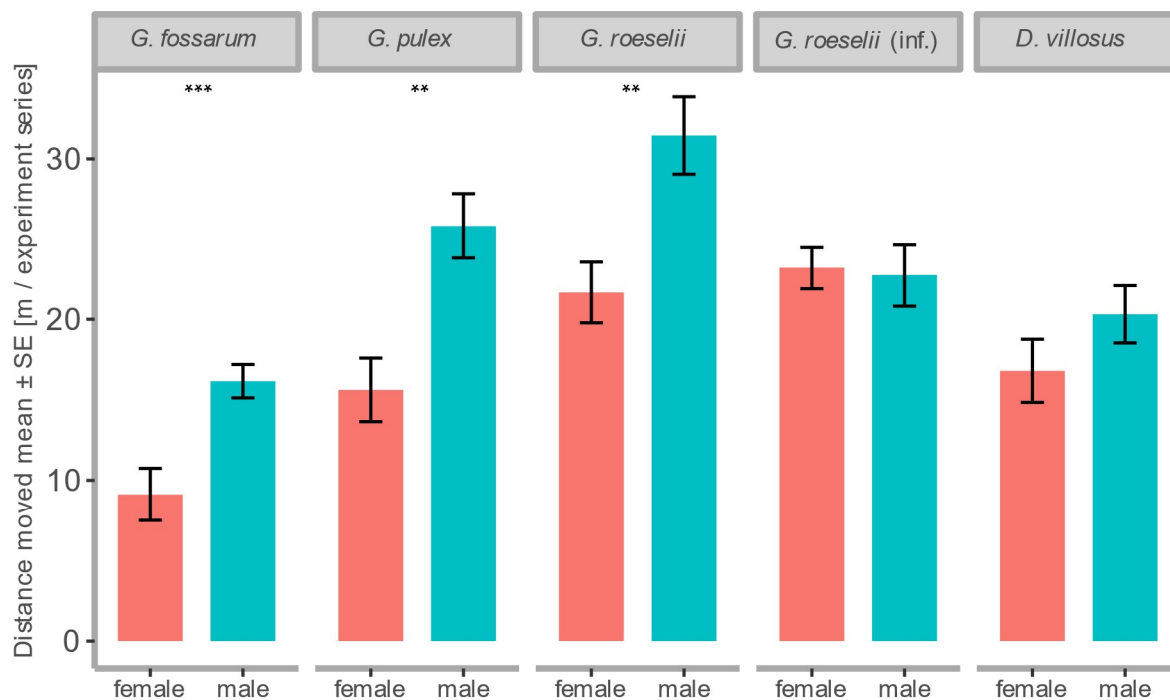
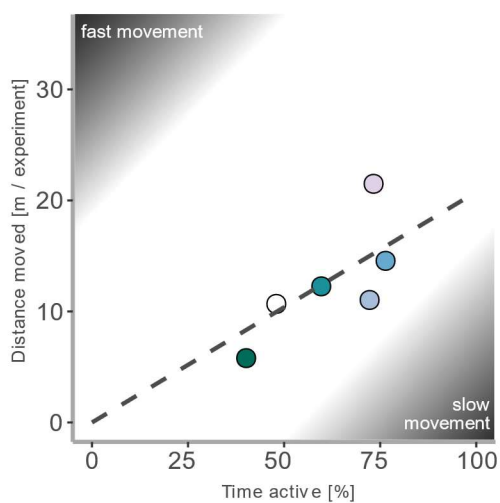
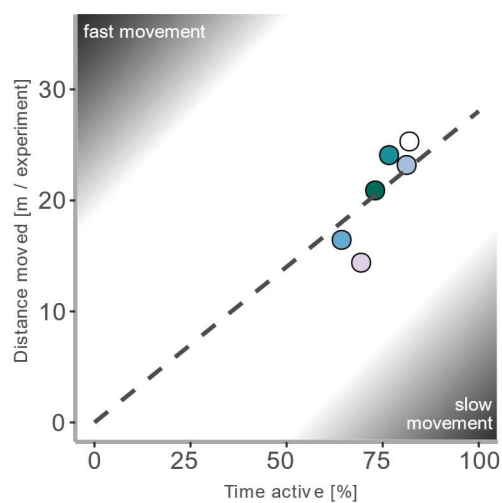
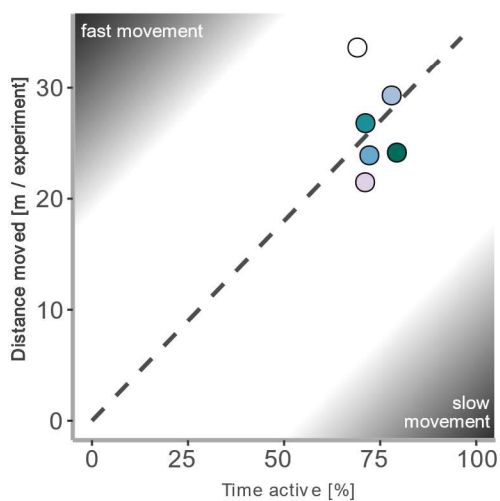
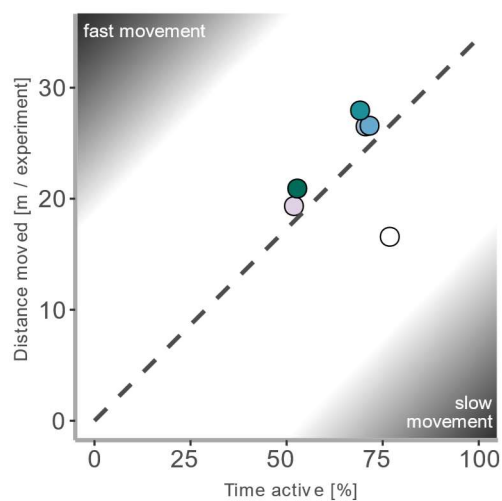
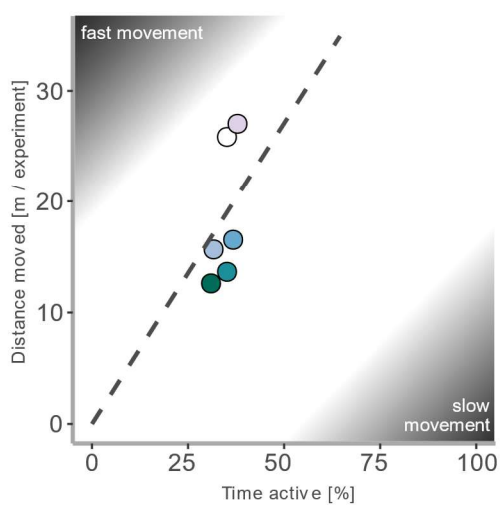


Figure S 2: Sex specific behavior four amphipod species and infected *G. roeselii* given as total distance moved per experiment. Values are based on estimated marginal means from single species GLMs. Error bars show the standard error. Asterisks indicate significant deviations between the sexes, based on Tukey's HSD post hoc test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

a) *G. fossarum*b) *G. pulex*c) *G. roesellii* (uninfected)d) *G. roesellii* (infected)e) *D. villosus*Concentration [$\mu\text{g} / \text{L}$]

- 0
- 0.32
- 1.04
- 3.31
- 10.6
- 33.92

Figure S 3 Combining total distance moved and time spent active of the thiacloprid treatments for the different amphipod species used. The trend line, forced to intersect the y-axis at $y = 0$, illustrates the linear association between the two variables and indicates a shift from fast movement to slow movement.

Table S 1 Overview of individuals in each experiment series. Total numbers, numbers of females and males used, and sex-ratio within the species.

| | | total | female | male | Sex-ratio (male:female) |
|----------------------------------|------------|--------------|------------------|-------------------|----------------------------|
| <i>G. fossarum</i> | NC | 31 | 10 | 21 | 2.1 |
| | 0.32 µg/L | 32 | 10 | 22 | 2.2 |
| | 1.04 µg/L | 32 | 6 | 26 | 4.3 |
| | 3.31 µg/L | 32 | 12 | 20 | 1.7 |
| | 10.6 µg/L | 32 | 14 | 18 | 1.3 |
| | 33.92 µg/L | 32 | 11 | 21 | 1.9 |
| | | 191 | 63 | 128 | 2.0 |
| <i>G. pulex</i> | NC | 32 | 17 | 15 | 0.9 |
| | 0.32 µg/L | 32 | 19 | 13 | 0.7 |
| | 1.04 µg/L | 32 | 16 | 16 | 1.0 |
| | 3.31 µg/L | 32 | 12 | 20 | 1.7 |
| | 10.6 µg/L | 32 | 17 | 15 | 0.9 |
| | 33.92 µg/L | 32 | 17 | 16 | 0.9 |
| | 192 | 98 | 95 | 1.0 | |
| <i>G. roeselii</i> | NC | 32 | 24 | 8 | 0.3 |
| | 0.32 µg/L | 33 | 17 | 16 | 0.9 |
| | 1.04 µg/L | 32 | 17 | 15 | 0.9 |
| | 3.31 µg/L | 32 | 17 | 15 | 0.9 |
| | 10.6 µg/L | 32 | 19 | 13 | 0.7 |
| | 33.92 µg/L | 32 | 20 | 12 | 0.6 |
| | 193 | 114 | 79 | 0.7 | |
| <i>G. roeselii</i> (infected) | NC | 32 | 19 | 13 | 0.7 |
| | 0.32 µg/L | 31 | 15 | 16 | 1.1 |
| | 1.04 µg/L | 32 | 21 | 11 | 0.5 |
| | 3.31 µg/L | 32 | 21 | 11 | 0.5 |
| | 10.6 µg/L | 32 | 16 | 6 | 0.4 |
| | 33.92 µg/L | 32 | 23 | 9 | 0.4 |
| | 191 | 115 | 66 | 0.6 | |
| | | total | <i>P. laevis</i> | <i>P. minutus</i> | Mean count |
| | | 596 | 554 | 42 | 3.1 |
| <i>D. villosus</i> | NC | 32 | 15 | 17 | 1.1 |
| | 0.32 µg/L | 32 | 14 | 18 | 1.3 |
| | 1.04 µg/L | 32 | 15 | 17 | 1.1 |
| | 3.31 µg/L | 31 | 17 | 14 | 0.8 |
| | 10.6 µg/L | 32 | 10 | 22 | 2.2 |
| | 33.92 µg/L | 32 | 16 | 16 | 1.0 |
| | 191 | 87 | 104 | 1.2 | |
| total | 958 | 477 | 472 | 1.2 | |

Table S 2 Results of independent GLMs of the four local amphipod species and infected *G. roeselii*. Endpoints used were total distance moved [m] (TDM) and time active [%] (TA). Significant results are highlighted in bolt.

| Dependent variable | Independent variables | <i>G. fossarum</i> | | | <i>G. pulex</i> | | | <i>G. roeselii</i> | | | <i>G. roeselii</i> (infected) | | | <i>D. villosus</i> | | |
|-----------------------------|-----------------------|--------------------|--------------|--------------|-----------------|---------|--------------|--------------------|-------------|--------------|-------------------------------|-------------|--------------|--------------------|-------------|--------------|
| | | Df | F value | P | Df | F value | P | Df | F value | P | Df | F value | P | Df | F value | P |
| a) Total distance moved [m] | concentration | 5 | 7.19 | 0.000 | 5 | 2.30 | 0.046 | 5 | 1.60 | 0.162 | 5 | 2.10 | 0.068 | 5 | 3.77 | 0.003 |
| | sex | 1 | 10.88 | 0.001 | 1 | 10.06 | 0.002 | 1 | 8.01 | 0.005 | 1 | 0.49 | 0.486 | 1 | 1.64 | 0.202 |
| | size | 1 | 0.24 | 0.626 | 1 | 0.88 | 0.349 | 1 | 0.05 | 0.820 | 1 | 0.12 | 0.726 | 1 | 2.98 | 0.086 |
| | concentration:sex | 5 | 1.26 | 0.285 | 5 | 0.55 | 0.739 | 5 | 1.20 | 0.311 | 5 | 2.13 | 0.064 | 5 | 0.90 | 0.483 |
| | Residuals | 178 | | | 179 | | | 180 | | | 178 | | | 178 | | |
| b) Time active [%] | concentration | 5 | 8.23 | 0.000 | 5 | 2.59 | 0.027 | 5 | 0.65 | 0.662 | 5 | 3.07 | 0.011 | 5 | 0.34 | 0.888 |
| | sex | 1 | 0.24 | 0.624 | 1 | 1.84 | 0.177 | 1 | 1.48 | 0.225 | 1 | 0.24 | 0.623 | 1 | 1.20 | 0.275 |
| | size | 1 | 0.13 | 0.720 | 1 | 2.55 | 0.112 | 1 | 0.21 | 0.647 | 1 | 2.20 | 0.140 | 1 | 1.08 | 0.301 |
| | concentration:sex | 5 | 1.91 | 0.095 | 5 | 0.91 | 0.475 | 5 | 1.65 | 0.149 | 5 | 1.63 | 0.154 | 5 | 0.90 | 0.484 |
| | Residuals | 178 | | | 179 | | | 181 | | | 177 | | | 178 | | |

Table S 3 Results of pairwise comparison from Tukey's HSD post hoc tests test for each amphipod species and infected *G. roeselii*.

| TDM [m] | contrast | estimate | SE | df | t.ratio | p.value | TA [%] | contrast | estimate | SE | df | t.ratio | p.value |
|----------------------------------|-----------------|---------------|-------------|------------|--------------|--------------|----------------------------------|-----------------|---------------|-------------|------------|--------------|--------------|
| <i>G. fossarum</i> | NC - 0.32 µg/L | -10.82 | 2.92 | 178 | -3.70 | 0.004 | <i>G. fossarum</i> | NC - 0.32 µg/L | -25.31 | 7.37 | 178 | -3.43 | 0.010 |
| | NC - 1.04 µg/L | -0.36 | 3.19 | 178 | -0.11 | 1.000 | | NC - 1.04 µg/L | -24.28 | 8.06 | 178 | -3.01 | 0.035 |
| | NC - 3.31 µg/L | -3.88 | 2.86 | 178 | -1.35 | 0.754 | | NC - 3.31 µg/L | -28.43 | 7.22 | 178 | -3.94 | 0.002 |
| | NC - 10.6 µg/L | -1.58 | 2.85 | 178 | -0.56 | 0.994 | | NC - 10.6 µg/L | -11.70 | 7.18 | 178 | -1.63 | 0.581 |
| | NC - 33.92 µg/L | 4.88 | 2.90 | 178 | 1.68 | 0.545 | | NC - 33.92 µg/L | 7.85 | 7.32 | 178 | 1.07 | 0.892 |
| <i>G. pulex</i> | NC - 0.32 µg/L | 10.92 | 4.04 | 179 | 2.70 | 0.080 | <i>G. pulex</i> | NC - 0.32 µg/L | 12.56 | 6.52 | 179 | 1.93 | 0.389 |
| | NC - 1.04 µg/L | 2.12 | 3.95 | 179 | 0.54 | 0.995 | | NC - 1.04 µg/L | 0.71 | 6.37 | 179 | 0.11 | 1.000 |
| | NC - 3.31 µg/L | 8.87 | 4.08 | 179 | 2.17 | 0.257 | | NC - 3.31 µg/L | 17.63 | 6.58 | 179 | 2.68 | 0.085 |
| | NC - 10.6 µg/L | 1.23 | 3.97 | 179 | 0.31 | 1.000 | | NC - 10.6 µg/L | 5.28 | 6.40 | 179 | 0.83 | 0.962 |
| | NC - 33.92 µg/L | 4.41 | 3.98 | 179 | 1.11 | 0.877 | | NC - 33.92 µg/L | 8.91 | 6.41 | 179 | 1.39 | 0.734 |
| <i>G. roeselii</i> | NC - 0.32 µg/L | 12.23 | 4.79 | 180 | 2.55 | 0.115 | <i>G. roeselii</i> (infected) | NC - 0.32 µg/L | -2.01 | 7.32 | 181 | -0.27 | 1.000 |
| | NC - 1.04 µg/L | 4.41 | 4.83 | 180 | 0.91 | 0.943 | | NC - 1.04 µg/L | -8.91 | 7.36 | 181 | -1.21 | 0.832 |
| | NC - 3.31 µg/L | 9.80 | 4.83 | 180 | 2.03 | 0.330 | | NC - 3.31 µg/L | -3.11 | 7.37 | 181 | -0.42 | 0.998 |
| | NC - 10.6 µg/L | 6.90 | 4.86 | 180 | 1.42 | 0.714 | | NC - 10.6 µg/L | -2.10 | 7.38 | 181 | -0.28 | 1.000 |
| | NC - 33.92 µg/L | 9.55 | 4.91 | 180 | 1.94 | 0.379 | | NC - 33.92 µg/L | -10.28 | 7.50 | 181 | -1.37 | 0.745 |
| <i>G. roeselii</i> (infected) | NC - 0.32 µg/L | -2.76 | 3.52 | 178 | -0.79 | 0.970 | <i>G. roeselii</i> | NC - 0.32 µg/L | 24.94 | 7.06 | 177 | 3.53 | 0.007 |
| | NC - 1.04 µg/L | -9.94 | 3.58 | 178 | -2.77 | 0.066 | | NC - 1.04 µg/L | 6.30 | 7.19 | 177 | 0.88 | 0.952 |
| | NC - 3.31 µg/L | -10.00 | 3.59 | 178 | -2.78 | 0.065 | | NC - 3.31 µg/L | 5.27 | 7.21 | 177 | 0.73 | 0.978 |
| | NC - 10.6 µg/L | -11.38 | 4.01 | 178 | -2.84 | 0.056 | | NC - 10.6 µg/L | 7.72 | 8.06 | 177 | 0.96 | 0.930 |
| | NC - 33.92 µg/L | -4.35 | 3.82 | 178 | -1.14 | 0.865 | | NC - 33.92 µg/L | 24.07 | 7.67 | 177 | 3.14 | 0.024 |
| <i>D. villosus</i> | NC - 0.32 µg/L | -1.19 | 4.52 | 178 | -0.26 | 1.000 | <i>D. villosus</i> | NC - 0.32 µg/L | -2.75 | 6.74 | 178 | -0.41 | 0.999 |
| | NC - 1.04 µg/L | 10.18 | 4.49 | 178 | 2.27 | 0.212 | | NC - 1.04 µg/L | 3.48 | 6.69 | 178 | 0.52 | 0.995 |
| | NC - 3.31 µg/L | 9.32 | 4.53 | 178 | 2.06 | 0.316 | | NC - 3.31 µg/L | -1.62 | 6.76 | 178 | -0.24 | 1.000 |
| | NC - 10.6 µg/L | 12.20 | 4.67 | 178 | 2.61 | 0.099 | | NC - 10.6 µg/L | -0.02 | 6.96 | 178 | 0.00 | 1.000 |
| | NC - 33.92 µg/L | 13.24 | 4.49 | 178 | 2.95 | 0.042 | | NC - 33.92 µg/L | 4.14 | 6.70 | 178 | 0.62 | 0.990 |

Table S 4 CRED reporting recommendations, to be used together with the accompanying guidance (Moermond et al., 2016). Overview table that gives targeted information about the method used and the results.

| Number | | Reported Yes/No | Reported in section | Comments |
|--------|--|-----------------|------------------------|---|
| 1 | General information | | | |
| a | Purpose of study | Yes | Intro | |
| b | Description of endpoints | Yes | Intro | |
| 2 | Test setup | | | |
| a | Performed according to standard/modified standard (e.g., OECDa, US EPAb) | No | non-standard study | |
| b | Performed according to Good Laboratory Practices (GLP) | No | | |
| c | Description of used control(s): negative control, solvent control, positive control | Yes | 2.4 Experimental setup | |
| d | Control(s) mortality, growth, morbidity and other observed non-standard effects like behavior and coloring | | Whole article | |
| e | Comparison to validity criteria (e.g. control survival, growth) from appropriate standard test method | No | | Need to be established |
| 3 | Test compound | | | |
| a | Identification (e.g. name, CAS-number, specify if the salt or the base is tested) | Yes | 2.5 Test substances | |
| b | Physico-chemical characteristics that may influence the behavior of the compound during the study (e.g. solubility, volatility, stability (hydrolysis, photolysis, degradation), solubility, log KOW, degradability, adsorption) | No | | Not provided due to shortening of the exposure period |
| c | Source | Yes | 2.5 Test substances | |
| d | Purity in % | Yes | 2.5 Test substances | |
| e | Composition of product formulation, and presence of impurities | | | |
| 4 | Test organism | | | |
| a | Scientific name | Yes | 2.1 Study species | <i>Gammarus fossarum</i> , <i>Gammarus pulex</i> , <i>G. roeselii</i> (plus potential acanthocephalan parasites), <i>Dikerogammarus villosus</i> |
| b | Body weight, length | Yes | length | Supplemental Dataset |

| | | | | |
|---|--|-----|---|---|
| c | Age/life-stage | Yes | 2.1 Study species | only mature individuals with fully developed sexual characteristics were used |
| d | Growth/reproductive condition | No | | |
| e | Sex | Yes | 2.4 Experimental setup; Table including Sex-ratio m:f | S 1 |
| f | Strain, clone | Yes | 2.2 Molecular identification | species/field individuals |
| g | Source, including possible pre-exposure for field-collected species | Yes | 2.1 Study species; Discussion | 4.WWTP, agricultural fields |
| h | Culture handling | Yes | 2.3 Sampling sites and culture handling | |
| 5 | Exposure conditions | | | |
| a | Exposure schedule (static, semi static, flow through system, other) and flow-rate (flow-through systems) or renewal time (semi-static systems) | Yes | 2.4 Experimental setup; | previous study: Soose et al. (2023) |
| b | Open or closed system | Yes | 2.4 Experimental setup; | previous study: Soose et al. (2023) |
| c | Test medium composition and source of test water (e.g., well water, deionised water, tap water) | Yes | 2.3 Sampling sites and culture handling; 2.4 Experimental setup | |
| d | Temperature, and time-points for measuring | Yes | 2.4 Experimental setup | |
| e | pH, and time-points for measuring | No | see Table S 5 | |
| f | Hardness of water, and time-points for measuring | No | see Table S 5 | |
| g | Conductivity, and time-points for measuring | No | see Table S 5 | |
| h | Dissolved oxygen content, and time-points for measuring | No | see Table S 5 | |
| i | Light intensity and quality (source and homogeneity), light/dark conditions | Yes | 2.4 Experimental setup | |

| | | | | |
|---|---|----------------|--|-----------------|
| j | Feeding protocols, food composition | Yes | 2.3 Sampling sites and culture handling | |
| k | Material and volume of aquarium/container and other equipment in contact with test organisms and test substance | Yes | 2.4 experimental setup | |
| l | Use of sand or sediment, and its characteristics (total organic carbon (TOC), particle size, etc.) | No | | |
| m | Preparation of stock solutions, including solvent concentrations in test water and controls | Yes | 2.5 Test substance | |
| n | Nominal concentrations of test substance | Yes | 2.5 Test substance | |
| o | Measured concentrations of test substance, and time-points for measuring | Yes | 2.5 Test substance | |
| p | Analytical method: description of method, including limit of detection (LOD), limit of quantification (LOQ) | Yes | 2.5 Test substance | |
| q | Exposure duration and total test duration | Yes | 2.4 Experimental setup | |
| r | Time-points of observations for endpoints | Yes | 2.4 Experimental setup | |
| s | Results based on nominal or measured concentrations | Yes | 3. Results | nominal |
| t | Biomass loading (biomass per liter) | No | | |
| 6 | Statistical Design and Biological Response | | | |
| a | Number of replicates for control(s) and test concentrations; setup of replicates (avoid pseudo-replication) | Yes | 2.4 Experimental setup | |
| b | Number of organisms, or algal cell concentration, per replicate | Yes | 2.4 Experimental setup; Table S 1 | |
| c | Treatment design (e.g., block, randomized) | Yes | 2.4 Experimental setup | randomly chosen |
| d | Statistical method used | Yes | 2.7 Statistical analyses | |
| e | Biological response for each concentration | Yes | | |
| f | Dose-response observed | Yes | | |
| g | Statistically significant responses noted (e.g. ECx) | Yes | 3 Results - Behavioral response to chemical exposure; Figure 2 | |
| h | Significance level for NOEC and LOEC data (0.05 or less) | Not applicable | | |
| i | Estimation of variability for LCx and ECx data | Not applicable | | |
| j | Availability of raw data: through supplementary information, a website, or upon request. | Yes | | |

a OECD = Organisation for Economic Co-operation and Development; b US EPA = The United States
Environmental Protection Agency

References

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Table S 5 Water parameters of medium

Table S 5 Measured parameters of test medium in climate controlled room at animal holding facility

| | Medium <i>G. fossarum</i> | Medium <i>G. pulex</i> , <i>G. roeselii</i> , <i>D. villosus</i> |
|--------------------|--|--|
| pH | 6.6 | 8.2 |
| Conductivity | 254 μ S | 836 μ S |
| Total hardness | 4.2° dH; 80 mg/L CaCO ₃ | 11° dH; 200 mg/L CaCO ₃ |
| Carbonate hardness | 0.5° dH; 0.2mmol/L SBV; 12.2 mg/L HCO ₃ | 8° dH; 3.3 mmol/L SBV; 201.36 mg/L HCO ₃ |
| O ₂ | 9.5 mg/L; 93.8 % at 13°C | 9.89 mg/L; 96.2% at 13°C |