Acute Exposure to Treated Wastewater Alters Swim Behavior in an Ecologically Relevant Zooplankton (Daphnia magna)

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Objective: Quantify behavioral changes in Daphnia that occur over time after exposure to various levels of treated wastewater

1. Background Information

1a. Pharmaceuticals & Personal Care Products as Contaminants
- Pharmaceuticals and personal care products (PPCPs) enter the environment through wastewater treatment plant (WWTP) effluent (Daughton, 2007)
- PPCPs are detected at small concentrations in surface waters worldwide
- Even at low levels, PPCPs alter behavior and physiology of aquatic organisms. This could lead to population collapses (Kidd et al, 2007)
- 57 PPCPs were detected in effluent from a WWTP in Burlington, VT (Vatovec et al, 2015)

1b. Daphnia as Experimental Organisms
- Daphnia (Fig. 1), planktonic crustaceans, are essential to freshwater ecosystem food webs (Fig. 8)
- Daphnia are widely used in water quality testing due to their sensitivity to pollutants
- Behavioral measurements indicate sublethal effects of chemical exposure on Daphnia (Zein et al, 2014)

2. Methods

2a. Effluent Sampling and Concentrations
- 500 mL of effluent sampled from WWTP in Burlington, VT (Fig. 2)
- Daphnia randomly assigned to one of three exposure conditions:
  1. Control (n=37; dechlorinated water)
  2. Environmentally Low (n=36; 1mL effluent per 150mL dechlorinated water)
  3. Environmentally High (n=39; effluent only)

2b. Experimental Design
- Subjects placed in 3mL wells (Fig. 3, 4). Videos recorded from above (Fig. 3) 0, 3, 6, and 24h later
- Used 2D motion tracking tool in Image Pro Premier software (Media Cybernetics, Fig. 4) to measure:
  1. Total distance travelled (mm)
  2. Average velocity (mm/s)
  3. Average change in angle (degree)

- Split-plot ANOVAs tested for differences in behavioral outcomes between groups and over time
- Image taken within Image Pro Premier software demonstrating how measurements were obtained using 2D motion tracking tool. Colored lines are tracks that highlight the movement of an individual over time

3. Results

3a. Total Distance Travelled (mm)
- Decrease over time in all groups (Fig. 5)
- Main effect of time (F[1,112] = 24.369, p < 0.001), but not group (F[2,112] = 1.514, p = 0.225)
- Time x group interaction was significant (F[2,112] = 4.089, p = 0.019)

3b. Average Velocity (mm/s)
- Decrease over time in all groups (Fig. 6)
- Main effect of time (F[1,112] = 7.462, p < 0.001), but not group (F[2,112] = 2.562, p = 0.082)
- Time x group interaction was not significant (F[2,112] = 1.436, p = 0.200)

3c. Average Change in Angle (degree)
- Increase over time in all groups (Fig. 7)
- Main effect of time (F[1,112] = 11.447, p < 0.001), but not group (F[2,112] = 0.320, p = 0.727)
- Time x group interaction was not significant (F[2,112] = 0.595, p = 0.553)

4. Conclusions
- Overall, time affected all measurements (Fig. 5-7) and effluent affected several measurements of Daphnia swim behavior (Fig. 5-6)
- The effect of time on total distance travelled appears to have been mediated by exposure to effluent (Fig. 5)
- The effect of time on average velocity was not dependent on group, yet subjects in the control group appeared to experience the greatest decline in velocity (Fig. 6)
- High concentrations of stimulatory chemicals (ex. caffeine) in the effluent could have produced such results
- Altered swim behavior could affect other important behavioral processes
- If individual fitness is negatively affected, the fitness of the population may be impacted. This could have consequences on food web dynamics (Fig. 6)
- The length of this study should be extended to test the chronic, and generational, effects of effluent exposure from multiple WWTPs

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