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The joint effects of salt and 6PPD contamination on a freshwater herbivore

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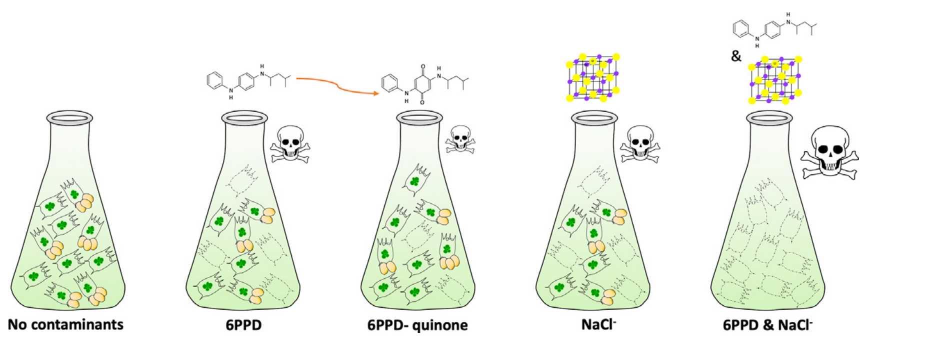
HIGHLIGHTS

- Road salt and 6PPD have negative effects on reproduction and survival of rotifers.
- The presence of one contaminant, enhances the negative effect of the other.
- For rotifers, 6PPD is more harmful than the oxidized 6PPD-quinone.
- Detrimental levels of the pollutants are above detected levels in the field.

GRAPHICAL ABSTRACT



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ABSTRACT

Using sodium chloride (NaCl) for de-icing roads is known to have severe consequences on freshwater organisms when washed into water bodies. N-(1,3-dimethylbutyl)-*N′*-phenyl-*p*-phenylenediamine, also known as 6PPD, is an antiozonant mainly found in automobile tire rubber to prevent ozone mediated cracking or wear-out. Especially the ozonated derivate, 6PPD-quinone, which is washed into streams after storm events, has been found to be toxic for coho salmon. Studies on other freshwater organisms could not confirm those findings, pointing towards distinct species-specific differences. Storm events result in greater run-offs from all water-soluble contaminants into freshwater bodies, potentially enhancing the concentrations of both chloride and 6PPD during winter. Here we show that these two contaminants have synergistic negative effects on the population growth of the rotifer *Brachionus calyciflorus*, a common freshwater herbivore. Hence, while only high concentrations of 6PPD and even higher concentrations of 6PPD-quinone, beyond environmentally relevant concentrations, had lethal effects on rotifers, the addition of NaCl enhanced the sensitivity of the rotifers towards the application of 6PPD so that their negative effects were more pronounced at lower concentrations. Similarly, 6PPD increased the lethal effect of NaCl. Our results support the species-specific toxicity of 6PPD and demonstrate a synergistic effect of the antiozonant on the toxicity of other environmentally relevant stressors, such as road salt contamination.

1. Introduction

Salinization of freshwater ecosystems is an emerging threat worldwide (Cañedo-Argüelles et al., 2016, 2013; Corsi et al., 2010; Szklarek et al., 2022) with a usage that has tripled over the past 45 years (Hintz et al., 2021). Agricultural use, industrialisation, mining (Cunillera-Montcusí et al., 2022) and the application of sodium chloride (NaCl) for de-icing roads in winter (Schuler et al., 2017) can lead to chloride concentrations in freshwater ecosystems that are exceeding national limits year-round (Oswald et al., 2019). Run-off from winter maintained roads can lead to elevated salt concentrations in streams (Kaushal et al., 2018, 2005; Kelly et al., 2008) and lakes (Corsi et al., 2010; Dugan et al., 2017a, 2017b) close to urban areas. Increasing salinity levels heavily impact freshwater organisms, impairing growth and development (Godwin et al., 2003; Hassell et al., 2006; Johnson et al., 2015; Sarma et al., 2006; Thunqvist, 2004). The sensitivity of freshwater organisms to elevated salt concentrations appears

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to be species-specific (Arnott et al., 2020) and environment-dependent (Brown and Yan, 2015; Isanta-Navarro et al., 2021). However, in nature it is seldom only a single stressor but rather a variety of stressors affecting ecosystem functioning and organismal interactions (Weisse et al., 2013). Contamination with harmful chemicals and micro-plastics has been studied increasingly in past decades. Tire wear particles contain both, harmful chemicals and micro-plastics, that have harmful effects on different organisms (Halle et al., 2021; Mantecca et al., 2009; Wik and Dave, 2009). N-(1,3-dimethylbutyl)-*N′*-phenyl-*p*-phenylenediamine, also known as 6PPD, is an antiozonant mainly found in tire rubber to prevent cracking or wear-out associated with ozone. Recently a study on coho salmon (*Oncorhynchus kisutch*) found that a derivate of 6PPD, 6PPD-quinone, caused acute mortality in the population after exposure to urban run-off (Tian et al., 2021). Given the large effect on salmon and importance of those findings, other studies have looked on the effect of 6PPD and its quinone on other freshwater organisms (Hiki et al., 2021; Varshney et al., 2021). The studies could not confirm the findings of acute lethal toxicity on zebrafish (*Danio rerio*), Japanese medaka (*Oryzias latipes*), water flea (*Daphnia magna*), and amphipod (*Hyalella azteca*) (Hiki et al., 2021). Only at non environmentally relevant concentrations of 6PPD and its quinone (10 and 25 μg/L) the behaviour of zebrafish larvae was negatively affected (Varshney et al., 2021).

6PPD has been found in roadway runoff, tire rubber leachates and road dust (Huang et al., 2021; Klöckner et al., 2021; Peter et al., 2018), ultimately ending up in freshwater ecosystems. Particularly freshwater ecosystems in urban areas, and especially during winter, might therefore be affected by both, 6PPD and salt contamination. In this study we investigated the joint effects of salt (NaCl) (0–2 g/L) and 6PPD (0–1000 μg/L) contamination in a fully factorial design on the freshwater herbivore *Brachionus calyciflorus*. Rotifers are invertebrates that constitute a major part of freshwater zooplankton and have developed into model organisms for evolutionary biology, ecology and toxicology. While the majority of environmental stressor assessments typically is studied on the freshwater keystone herbivore *Daphnia*, it is also important to know the effect of contaminants on other zooplankton species. Among metazoans, rotifers have one of the highest reproductive rates. By obtaining high population densities they have the potential to dominate zooplankton communities, serving as an important link between the microbial community and higher trophic levels in freshwater ecosystems (Rico-Martínez et al., 2016). Identifying taxa specific differences is crucial for predictions about potential changes in food web dynamics upon contamination.

Besides studying the individual effects of 6PPD and road salt on the population growth of *B. calyciflorus*, our experimental set up further allowed us to test whether the presence of one stressor enhances the negative effects of the other stressor on the population growth of *B. calyciflorus*. We also compared the effects of 6PPD and 6PPD-quinone to identify if the antiozonant itself or the quinone causes greater effects on rotifers. Given the pronounced effects of 6PPD-quinone of coho salmon we hypothesized that the quinone will have greater negative effects on the rotifers than the antiozonant itself. This study contributes to our understanding of the toxicity of 6PPD and 6PPD-quinone and for the first time, investigates the relationship with another highly relevant anthropogenic stressor, salt contamination.

2. Material and methods

2.1. Culture conditions

Experiments were conducted with the herbivorous rotifer *Brachionus calyciflorus* (kindly provided by Prof. Dr. Lutz Becks, University of Konstanz, Germany) and the phytoplankton *Monoraphidium minutum* [SAG 243-1; Culture Collection of Algae, University of Göttingen, Göttingen, Germany]. Stock cultures of *B. calyciflorus* and *M. minutum* were reared in sterile and vitamin-supplemented Woods Hole culture medium (WC) (Guillard and Lorenzen, 1972) and kept in batch cultures at 20 °C with weekly substitutions of fresh medium or food suspension.

2.2. Population growth experiments

The impact of salt (NaCl) and 6PPD on the population dynamics of *B. calyciflorus* was assessed in a full factorial semi-continuous population growth experiment comprising five different salt (0, 0.25, 0.5, 1 and 2 g/L) and 6PPD (0, 125, 250, 500 and 1000 μg/L; obtained from TCI Chemicals, Product number: D3331, CAS RN: 793-24-8, purity: > 98%) concentrations. Salt concentrations were chosen to cover a wide range of reported values for lakes and streams (see Hintz et al., 2021, 0–1 g Cl^-/L) and were additionally based on previous studies, investigating the effect of salt on rotifers (see Sarma et al., 2006; 0–4.5 g NaCl/L). To compare the impact of 6PPD with the impact of 6PPD-quinone on the population dynamics we additionally conducted an experiment that comprised 6PPD-quinone (obtained from: ASCA GmbH, Angewandte Synthesechemie, Berlin, Product number: 10243, Lot number: JB14077, purity: 98.9%) at a concentration of 1000 μg/L. Since 6PPD is solved in methanol, an increase in the concentration of 6PPD inevitably goes along with an increase in the concentration of methanol (positive covariation). To avoid such confounding effects, each treatment exhibited the same final methanol concentration (1 mL/L) which was achieved by adding additional methanol to the flasks after the 6PPD/methanol solution was added (for details see Supporting Information, Table S1). In order to evaluate whether the final methanol concentration itself had any effect on the rotifer and phytoplankton growth, we included a “no contaminant treatment” that did not contain any pollutants, i.e. methanol, additional salt or 6PPD.

For our experiment, we used 100-mL Erlenmeyer flasks that were sealed with cork plugs, which prevented a contamination of our experimental cultures but still allowed for a continuous exchange of O_2 and CO_2 between the inner and outer environment. Experiments were conducted in three climatic controlled chambers (Rubarth Apparate GmbH, Laatzen, Germany) at 20 °C under continuous light supply [120 ± 15 μmol photons (photosynthetically active radiation)/m^2s]. Cultures were kept in suspension by gentle shaking on a vibrating plate [GFL-3015, Gesellschaft für Labortechnik mbH].

At the beginning of the experiment, each flask was filled with 100 mL WC medium and inoculated with algae (OD 0.062 at 800 nm) and rotifers (5.25 Ind/mL). Each treatment was replicated three times, resulting into a total of 81 experimental units. Erlenmeyer flasks were randomly distributed across the three different climate chambers, with each climate chamber containing 27 flasks. The experiment ran for 12 days, 10 mL of the plankton suspension were sampled every day and the volume was replaced with fresh medium containing the treatment-specific amounts of pollutants. This results into a dilution rate of 0.1/day of each treatment. Samples were fixed in 1% Lugol's iodine solution after measuring the optical density of the suspension at 800 nm in a 5 mL cuvette (UVmini-1240, Shimadzu, Kyoto, Japan). Rotifers were counted from a subsample of the original 10-mL sample in a counting chamber for zooplankton under a light microscope (ZEISS Primovert or ZEISS Axio Observer A1), distinguishing between living and dead rotifers and eggs.

2.3. Data analysis

To evaluate the impact of salt and 6PPD on the population dynamics of rotifers, we estimated the maximum population growth rates based on the exponential (initial) growth phase of the observed population dynamics. For this we fitted a linear regression model to the logarithmic population densities of the adults for the days 1 to 6, by using the function *fitnlm*() from *MATLAB*. We further analyzed the combined and potentially interactive effects of salt and 6PPD on the maximum growth rates of the rotifers, by fitting a bivariate non-linear regression model (second-order polynomial) to the previously estimated values using the function *fit*() from *MATLAB*. The non-linear regression model reads as follows:

$$F(s,p) = c_{00} + c_{10}\bullet s + c_{01}\bullet p + c_{20}\bullet s^2 + c_{11}\bullet s\bullet p + c_{12}\bullet p^2$$

with s and p denoting the independent variables and thus concentrations of salt and 6PPD. The coefficient c_{00} represents the predicted maximum growth rate by the model when both stressors are absent. In contrast, c_{10} and c_{01} are the coefficients of the linear components of the regression model and thus represent the impact of the stressors at lower concentrations. In addition, c_{20} and c_{02} are the coefficients of the quadratic components for each individual stressor of the regression model and thus represent the impact of the stressors at higher concentrations. Finally, the interaction coefficient c_{11} of the regression model informs about whether the presence of one stressor affects the response of the dependent variable to the other stressor. Stressors may interact in different ways, they can be synergistic (larger combined affect than the sum of the individual effects), antagonistic (smaller combined affect than the sum of the individual effects) or additive (effects linearly add to each other) (Feld et al., 2016). In line with Ellis et al. (2017) and Mahon et al. (2019), we can interpret the different parameters of our regression model to quantify the additive and interactive effects of our two different stressors. While the coefficients c_{10}, c_{01}, c_{20} and c_{02} describe the independent linear and non-linear additive effects of our two different stressors, the coefficient c_{11} quantifies their interactive effect, which in our case is further to be considered as synergistic when c_{11} is negative and to be antagonistic when c_{11} is positive. Data analyses and plotting were performed using *MATLAB*, version 9.10 (MATLAB, 2019. version 9.6.0 (R2019a), Natick, Massachusetts: The MathWorks Inc., 2021).

3. Results and discussion

In the control treatment the rotifers started to reproduce quickly after inoculation, leading to a substantial increase in the number of living adults over time (Fig. 1a). As a result of this enhanced top-down control by the rotifers, the algae were unable to grow towards higher densities, experiencing even a substantial decline in their population size. The subsequent shortage of suitable food reduced the egg production of rotifers, increased the number of dead individuals and led to a collapse in the number of living adults towards the end of the experiment. The addition of the two contaminants, 6PPD and salt, strongly affected the population dynamics of the rotifers when compared to the control treatment (Figs. 1, 2, S1). While the lowest concentrations used for both contaminants hardly influenced the population growth of the rotifers (Figs. 2, S1), the further addition of salt and 6PPD caused a strong reduction in the maximum growth rate of the rotifers due to an enhanced mortality and a reduced fertility of the adults when the highest concentrations of both contaminants were applied (Figs. 1b, c; 2). These individual effects were independent from the presence or absence of the other contaminant. By exposing the population to both contaminants, the negative effects were more pronounced. At the highest concentration of both contaminants (6PPD = 1000 μg/L, salt = 2 g/L) the population exhibited negative growth after initial inoculation (Figs. 1d; 2). Only few living adults were found after day two. These results suggest a pronounced negative impact of salt and 6PPD on the rotifer population growth at higher concentrations. Our results further show that there is a negative interaction between both contaminants, pointing towards an amplifying and thus synergistic effect (Fig. 2). That is, in the presence of factor one, the negative effect of factor two is enhanced and vice versa. Organisms of freshwater ecosystems that are exposed to both elevated salt concentrations and 6PPD might be particularly vulnerable as the negative interaction implies a higher sensitivity or susceptibility of the rotifers to an increase in the concentration of a particular contaminant when the other contaminant is present as well. Likely this will be the case in urban areas during winter.

Recent results on coho salmon (*Oncorhynchus kisutch*) (Tian et al., 2021) suggests that a derivate of 6PPD, 6PPD-quinone, may have even more severe effects on the growth of natural populations. We therefore also investigated the impact of 6PPD-quinone on the growth of *B. calyciflorus*. In preliminary short-term experiments we found the effect of the 6PPD-quinone on the rotifers to be less pronounced than the effect of 6PPD, with some rotifers surviving at the highest concentration (10,000 μg/L). We therefore decided to perform the main experiment with 6PPD instead of the quinone. However, during our main experiment we included one quinone treatment (Fig. 1e), with the same concentration as the highest 6PPD treatment (1000 μg/L). In line with our preliminary results, a 6PPD-quinone concentration of 1000 μg/L hardly affected the growth of the rotifer population as the shape of the population dynamics of the 6PPD-quinone treatment (Fig. 1d) largely follows the one from the control (0 salt, 0 6PPD, 1 ml/L methanol) treatment (Fig. 1b).

While the rotifer abundance initially dropped to very low values at the highest 6PPD concentration of 1000 μg/L, their population size increased again towards the end of the experiment when the salt concentration was not exceeding 1 g/L (Figs. 1b, S1). Although a natural population might in principle be able to rapidly evolve resistance to a contaminant, in this experiment the population of *B. calyciflorus* is grown from a few asexually reproducing individuals. Therefore rapid adaptation appears to be unlikely, the observed pattern rather points towards the overall poor solubility and low stability of 6PPD in water, having a maximum solubility of 563 μg/L and a half-life of 5 h at 23 °C (Hiki et al., 2021). For this reason, 6PPD gets usually dissolved in ethanol (Varshney et al., 2021) or methanol (our study) before application. The poor solubility of 6PPD potentially led to an enhanced crystallization of 6PPD over time at higher concentrations (for further discussion please see Supporting Information, Figs. S2 and S3). Although we lack more quantitative information, the crystallization capacity of 6PPD is well known and has been used for purification in another study (Cibulková et al., 2009). A further reduction of the actual concentration of 6PPD in our treatments over time likely occurred due to the low stability of 6PPD in water, potentially degrading constantly towards 4-hydroxydiphenylamine (cf. Hiki et al., 2021). Hence, while the initial concentration seemed to have been sufficient enough to reduce the early population growth of *B. calyciflorus*, enhanced crystallization and degradation of 6PPD over time might have reduced its actual concentration and thus, the toxicity for *B. calyciflorus* towards the end of the experiment, potentially explaining the late regrowth of its population.

To evaluate whether methanol itself has an impact on the population dynamics, we included a no contaminant treatment. While the rotifers established a stable and high final population size in the no contaminant treatment, that was able to suppress the algal prey to very low densities at the end of the experiment (Fig. 1f), this strong top-down control seems to be disrupted by the addition of methanol in the control treatment (Fig. 1a). A potential explanation for this difference might be a strong bacterial growth in the control treatment that did not occur in the no contaminant treatment (for further discussion please see Supporting Information, Fig. S4). However, most of our analysis focusses on the first half of the experiment where the impact of methanol on the population dynamics was rather negligible. Furthermore, we accounted for this effect by keeping the methanol concentration the same in all treatments, enabling us to study the sole response of rotifers to increasing levels of 6PPD.

While the concentrations of 6PPD, the quinone and salt are beyond environmentally relevant concentrations, they were chosen to explore the full range of the effect on rotifers. Maximum concentrations of the quinone observed in rivers during storm events were 2.3 μg/L (Johannessen et al., 2021), 0.6 μg/L in stormwater of cold-climate Canadian cities and 0.08–0.37 μg/L were found in snowmelt (Challis et al., 2021). The maximum water solubilities of the 6PPD and the quinone have been found to be 563 and 67 μg/L at 23 °C, respectively (Hiki et al., 2021). While those concentrations have not been observed in natural environments yet, they are close to the concentrations used in our experiment. The differences in solubility and effect on organisms lead to the question, which contaminant might be potentially more harmful to natural systems, 6PPD or the oxidized quinone? Regularly measuring the concentrations of both, the original and oxidized form in natural ecosystems might help to understand which contaminant is found more frequently. The effect of both contaminants on aquatic organisms seems to be highly dependent on the species. So far, no other study, including ours, could confirm the striking effects that the quinone had on coho salmon (Tian et al., 2021) on any other freshwater organism (Hiki et al., 2021; Varshney et al., 2021). However, new car tires have been found to be less toxic than old car tires to the rainbow trout (*Oncorhynchus mykiss*) (Day et al., 1993). The family of Salmonidae has strict environmental requirements (cold and clean water) and is known to

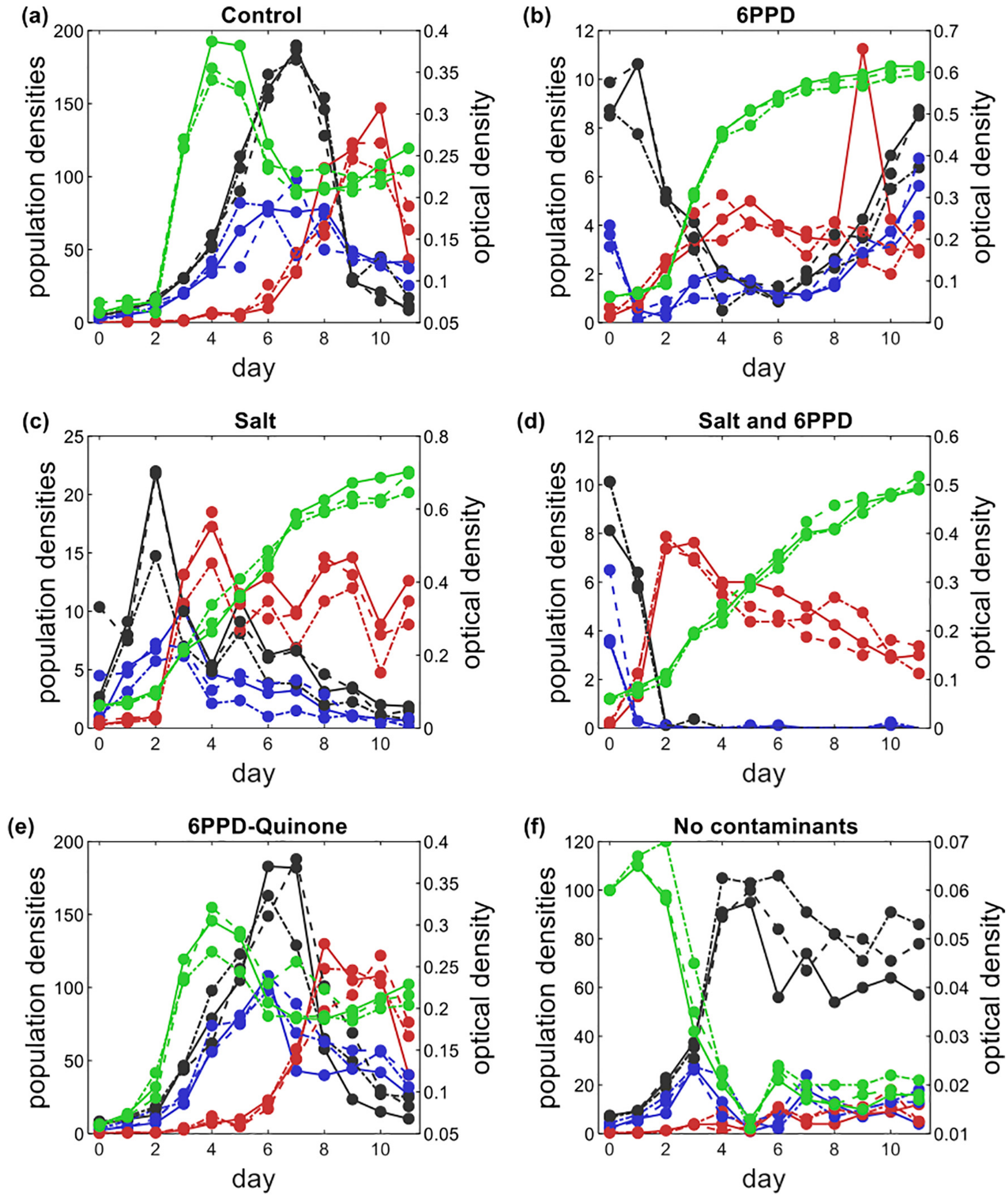

Fig. 1. The impact of methanol, 6PPD and 6PPD-quinone on the temporal dynamics of rotifers and algae (optical density). a) control treatment (1‰ methanol but no other substances), b) highest sole 6PPD treatment (1‰ methanol and 1000 μg/L 6PPD), c) highest sole salt treatment (1‰ methanol and 2 g/L NaCl) d) highest combined 6PPD and salt treatment (1‰ methanol, 1000 μg/L 6PPD and 2 g/L NaCl), e) 6PPD-quinone treatment (1‰ methanol and 1000 μg/L 6PPD-quinone) and f) no contaminant treatment (no methanol or other substances). With black = living adults, blue = eggs produced, red = dead individuals and green = optical (algal) densities. The dashed, dotted and solid lines represent the three replicates. For all results of the fully factorial experiment please see Supporting Information (Fig. S1).

be highly sensitive to contaminants and climate change (Williams et al., 2015). Those properties might explain why the striking effects of the quinone have only been found for the coho salmon so far. However, the potential importance of 6PPD and the quinone as contaminants is a relatively new topic. Potential detrimental effects on other freshwater organisms could be detected in future studies.

In contrast to the rather recent interest in studying the effect of 6PPD on aquatic ecosystems, years of research have shown that road salt (NaCl) negatively affects organisms at all trophic levels and that water quality guidelines do not offer sufficient protection (Hintz et al., 2022). However, in line with the first results of the impact of 6PPD on freshwater organisms, not only lethal thresholds for NaCl vary across species but also non-lethal concentrations that have an effect on survival, growth and reproduction (Hintz and Relyea, 2019). At the zooplankton level, most research has focused on the keystone herbivore *Daphnia* because of their critical role in the energy transfer within freshwater ecosystems (Arnott et al., 2020; Gonçalves et al., 2007; Hintz and Relyea, 2017; Martínez-Jerónimo and Martínez-Jerónimo, 2007; Sarma et al., 2006). In comparison, less is

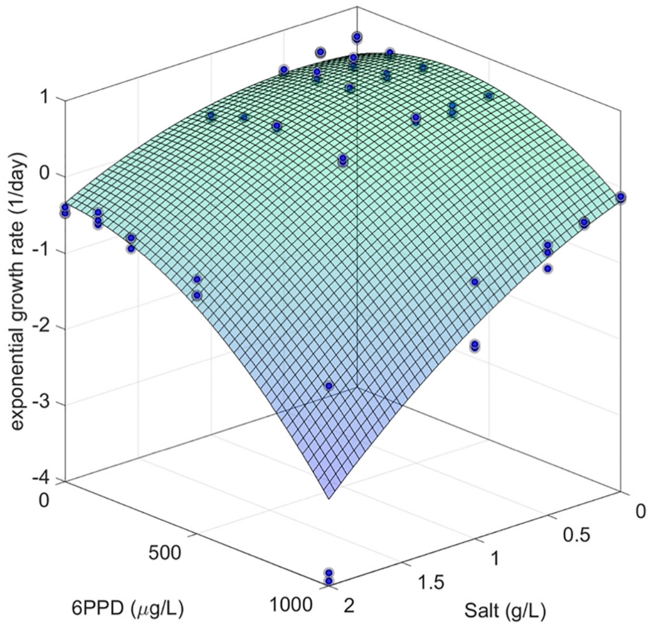

Fig. 2. Nonlinear regression model for maximum growth rates of adult rotifers (significant differences in bold). The coefficients (with 95% confidence bounds) are: $c_{00} = 0.38$ (0.12, 0.56), $c_{10} = 0.28$ (-0.09, 0.64), $c_{01} = 0.0017$ (0.00098, 0.0025), $c_{20} = -0.32$ (-0.48, -0.15), $c_{11} = -0.00098$ (-0.0012, -0.00072), $c_{02} = -0.0000023$ (-0.0000029, -0.0000016).

known about the salt tolerance of other zooplankton taxa such as rotifers and copepods (Hintz and Relyea, 2017 but see Sarma et al., 2006; Sinclair and Arnott, 2018; Stoler et al., 2017). The few studies that looked at rotifer salt tolerance in freshwater systems found great variation even within the same genus, with values ranging from 1 g NaCl/L to 4.5 g NaCl/L (Ferrando et al., 2019; Sarma et al., 2006). In the absence of contamination with 6PPD, salt addition hardly affected rotifer growth in our experiment for concentrations below or equal to 1 g NaCl/L. However, the mortality of *B. calyciflorus* was substantially enhanced at a salt concentration of 2 g NaCl/L. This suggests that salt negatively affects the growth of *B. calyciflorus* at concentrations between 1 and 2 g NaCl/L. Similar ranges are reported by Sarma et al. (2006) and Ferrando et al. (2019), with mild and strong reductions in population growth rates of *B. calyciflorus* at 1.5 g or 1.75 g NaCl/L and 2.75 g or 3 g NaCl/L, respectively. Hence, our observations fall within the range of rotifer salt tolerance that has been observed previously for this species. Future studies could investigate if elevated salt concentrations induce sexual reproduction in the rotifer populations (Lubzens et al., 1985; Snell, 1986) or if individuals are able to physiologically adapt to altered salinities in freshwater ecosystems. Both processes have the potential to enhance the tolerance of natural rotifer populations to salt contamination of water bodies. In contrast to our rotifer species *B. calyciflorus*, the salt tolerance of our phytoplankton species, *M. minutum*, is difficult to assess in the presence of rotifer grazing. While higher salt concentrations may directly and negatively affect the maximum growth rate of phytoplankton species, a simultaneous decrease of its grazer community may have a positive indirect effect on the phytoplankton growth through reduced grazing mortality. Nevertheless, at the highest salt concentration, when predation was low, an average optical density of about 0.65 was recorded after 12 days. This falls within the range of reported thresholds for nine different green algae, where all species were able to proliferate (Figler et al., 2019). Only at concentrations above 5 g NaCl/L, a significant inhibition of the phytoplankton species was observed (Figler et al., 2019). Our highest salt concentration of 2 g NaCl/L therefore will likely not have inhibited phytoplankton growth.

Finally, it is of general interest that *Daphnia* seem to be more sensitive towards salt contamination (Greco et al., 2021; Sarma et al., 2006) as well as 6PPD and the quinone. *Daphnia magna*, a species that often inhabits eutrophic, smaller ponds has been found to have 100% mortality upon 48 h exposure to 138 μg/L 6PPD and 46 μg/L of the quinone (Hiki et al., 2021). Different *Daphnia* species have been assessed in their tolerance to salt contamination. Depending on the species, reproduction decreased and mortality increased already at salt concentrations of 5 to 40 mg Cl/L (Arnott et al., 2020). *Daphnia* and rotifers are both freshwater herbivores, potentially utilizing similar food sources in many lake ecosystems. Given the higher tolerance of rotifers to both contaminants, a potential shift in zooplankton dynamics might be occurring in highly contaminated lakes. This in turn could have effects on the composition and structure of organisms that are feeding on rotifers or crustaceans and the energy transfer towards higher trophic levels.

4. Conclusion

This study demonstrates for the first time the joint effects of two environmentally relevant pollutants, salt and 6PPD, on a freshwater herbivore. Our results show that both pollutants have a negative effect on the population growth rate of the rotifer *Brachionus calyciflorus*. Additionally, the negative effect of one pollutant is enhanced in the presence of the other pollutant and vice versa. This suggests, that freshwater ecosystems and the inhabiting organisms close to urban areas, are particularly vulnerable to a contamination with harmful substances as they will frequently be exposed to run-off containing both pollutants, especially during winter. Furthermore, our findings indicate that 6PPD rather than the oxidized form, the 6PPD-quinone, is a threat to rotifer populations. However, concentrations that we found to cause detrimental effects on the rotifer population are above reported levels detected in the field.

CRediT authorship contribution statement

Toni Klauschies: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Jana Isanta-Navarro:** Conceptualization, Validation, Writing - original draft, Writing - review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Additional experimental details, materials, and methods, including photographs of experimental setup. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154675.

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