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Effects of conventionally treated and ozonated wastewater on the damselfly larva oxylipidome in response to on-site exposure

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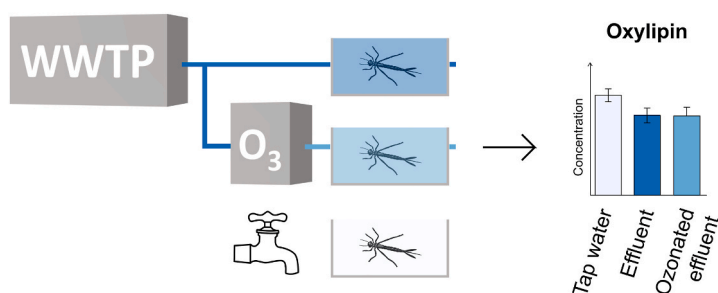[a] Department of Chemistry, Umeå University, SE 90187, Umeå, Sweden
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HIGHLIGHTS

- Average ozonation removal efficiency of 67% at an ozone dose of 0.49 g O3/g DOC.
- Oxylipins 12(13)-EpODE and 15(16)-EpODE were reduced in larvae exposed to conventionally treated wastewater.
- 15(16)-EpODE was also reduced in larvae exposed to ozonated wastewater.

GRAPHICAL ABSTRACT

ABSTRACT

Pharmaceutical residues discharged through insufficiently treated or untreated wastewater enter aquatic environments, where they may adversely impact organisms such as aquatic invertebrates. Ozonation, an advanced wastewater treatment technique, has been successfully implemented to enhance the removal of a broad range of pharmaceuticals, however diverse byproducts and transformation products that are formed during the ozonation process make it difficult to predict how ozonated wastewater may affect aquatic biota. The aim of this study was to investigate effects on fatty acid metabolites, oxylipins, in a common invertebrate species, damselfly larvae, after on-site exposure to conventional wastewater treatment plant (WWTP) effluent and additionally ozonated effluent at a full-scale WWTP. Subsequent ozonation of the conventionally treated wastewater was assessed in terms of i) removal of pharmaceuticals and ii) potential sub-lethal effects on the oxylipidome. Northern damselfly (*Coenagrion hastulatum*) larvae were exposed for six days in the treatment plant facility to either conventional WWTP effluent or ozonated effluent and the effects on pharmaceutical levels and oxylipin levels were compared with those from tap water control exposure. Ozonation removed pharmaceuticals at an average removal efficiency of 67% (ozone dose of 0.49 g O$_3$/g DOC). Of 38 pharmaceuticals detected in the effluent, 16 were removed to levels below the limit of quantification by ozonation. Levels of two oxylipins, 12(13)-EpODE and 15(16)-EpODE, were reduced in larvae exposed to the conventionally treated wastewater in comparison to the tap water control. 15(16)-EpODE was reduced in the larvae exposed to ozonated effluent in comparison to the tap

water control. One oxylipin, 8-HETE, was significantly lower in larvae exposed to conventional WWTP effluent compared to ozonated effluent. In conclusion, the study provides proof-of-principle that damselfly larvae can be used on-site to test the impact of differentially treated wastewater.

1. Introduction

Anthropogenic micropollutants, including pharmaceuticals, have been found at trace concentrations in the aquatic environment worldwide (Ternes, 1998; Heberer, 2002; Luo et al., 2014; Yang et al., 2017; Wilkinson et al., 2022). Pharmaceuticals cause physiological effects in living organisms and have raised concern regarding their accidental and potentially adverse effects on exposed biota (Ternes, 1998). Such effects of pharmaceuticals include acute and long-term toxicity (Wiles et al., 2020; Thoré et al., 2021), behavioural alterations (Brodin et al., 2014), antibiotic resistance of microorganisms (Levy and Marshall, 2004), endocrine disruption (Tyler and Susan, 2008), and accumulation in sediments and biota (Miller et al., 2018). Pharmaceutical residues enter the aquatic environment through insufficiently treated wastewater treatment plant (WWTP) effluents and untreated effluents (Zorita et al., 2009; Yang et al., 2017).

Advanced wastewater treatment techniques, including ozonation, have successfully been implemented to enhance the removal of a broad range of anthropogenic pollutants (Huber et al., 2005; Eggen et al., 2014). During ozonation, the reactive gas ozone (O_3) is used to oxidise, and thus transform, micropollutants in the water, either directly by a reaction with O_3 or secondarily by formation of hydroxyl radicals (Schindler Wildhaber et al., 2015). When applied on wastewater, ozonation can reduce micropollutant load by $> 80\%$ (Abegglen and Siegrist, 2012). However, there are conflicting findings on the residual toxicity as well as biological effects of ozonated wastewater. Some studies observed reduced toxicity of ozonated wastewater, for example, in terms of reduced estrogenicity in fish (An et al., 2008; Filby et al., 2010; Gunnarsson et al., 2009), and reduced genotoxic activity of bacteria, plant, and mammalian cells (Mišík et al., 2011). Other studies reported potentially adverse effects on exposed biota, for example increased reproduction of water flea *Ceriodaphnia dubia* (Blatchley et al., 1997), increased cytochrome P450 (CYP) induction in nematode *Caenorhabditis elegans* (Abbas et al., 2018), and increased reproduction and estrogenicity as well as decreased activity in fish (Pohl et al., 2018). No effects of ozonation were detected on enzymatic activities in the amphipod *Gammarus fossarum* (Wigh et al., 2017), neuroendocrinal effects in freshwater mussels *Elliptio complanata* (Gagné et al., 2011), as well as endocrine disruption in fish (Altmann et al., 2012) when compared to conventional WWTP effluent. Especially byproducts and transformation products formed during the oxidation process need to be assessed in terms of their biological effects (Eggen et al., 2014).

Due to the high complexity and variability, WWTP effluents are difficult to assess in terms of ecotoxicological risks for exposed organisms (Eggen et al., 2014; Kümmerer et al., 2019; Love et al., 2020). Biomarkers for sub-lethal effects in a sentinel organism can be used to assess potential biological effects of effluent exposure. One aquatic invertebrate species, larval damselflies, are a suitable group for a sentinel organism due to their central position in food webs and wide distribution (Jonsson et al., 2014). In addition, effects of exposure to a broad range of anthropogenic pollutants, including heavy metals, pesticides, and pharmaceuticals, have been previously studied in damselfly larvae providing baseline information facilitating effluent-testing (Boroń and Mirosławski, 2009; Janssens and Stoks, 2013; Jonsson et al., 2014).

As endpoints, biochemical biomarkers of sub-lethal effects such as the enzymes catalase (CAT), cholinesterase (ChE), and cytochrome P450 (CYP), have been measured in various aquatic species to establish their ability to reflect pollution in the aquatic environment (Sarkar et al., 2006; Wigh et al., 2017; Rodrigues et al., 2019). To evaluate the residual

toxicity of wastewater treated with ozonation and other additional wastewater treatment techniques, vitellogenin (VTG) has been used as a biomarker for estrogenicity in fish (An et al., 2008; Gunnarsson et al., 2009; Filby et al., 2010). A new group of potential biochemical biomarkers are oxylipins (the oxylipidome). Oxylipins have numerous signaling functions in both vertebrate and invertebrate species (Heckmann et al., 2008; Dennis and Norris, 2015). As fatty acid metabolites, oxylipins derive from precursor polyunsaturated fatty acids (PUFAs) by enzymatic oxidation via cyclooxygenase (COX), lipoxygenase (LOX) and CYP pathways. The COX enzyme is inhibited by non-steroidal anti-inflammatory drugs (NSAID) (Willenberg and Ostermann, 2015), one of the most commonly prescribed classes of medication for pain and inflammation in humans. Since 5–10% of all medications prescribed each year belong to the class of NSAID targeting enzymes in the oxylipin cascade, their presence in WWTP effluent is of major concern in terms of effects on aquatic organisms. Other pharmaceuticals and micropollutants that are not sufficiently removed during conventional wastewater treatment strategies might also contribute to exposure effects. Accordingly, levels of a group of COX-derived oxylipins (prostaglandins) were reduced in fish exposed to WWTP effluent (David et al., 2017). Furthermore, exposure to psychiatric drugs altered oxylipin levels in the crustacean *Daphnia magna* (Fuchs et al., 2018). Oxylipins are promising biochemical markers for sub-lethal effects because they may indicate impacts on various enzymatic pathways and thus may better illustrate the response of an organism to WWTP effluent exposure than other biomarkers of exposure.

In previous lab-scale experiments we found that oxylipins were responsive to WWTP effluent exposure in damselfly larvae (Späth et al., 2020, 2021). Moreover, oxylipin levels depended on the extent of treatment, i.e., less oxylipins were affected in damselfly larvae exposed to effluent treated with biochar and ozonated effluent compared to conventional WWTP effluent (Späth et al., 2020).

The aim of the present study was to investigate effects of WWTP effluent exposure on oxylipin levels on-site a full-scale WWTP under the hypothesis that ozonation results in removal of pollutants impacting the oxylipidome. To that end, the removal efficiency of the ozonation step was investigated in terms of i) pharmaceutical content and ii) biological sub-lethal effects on the oxylipidome. Northern damselfly (*Coenagrion hastulatum*) larvae were exposed for six days in the treatment plant facility to either conventional WWTP effluent or ozonated effluent and the effects on pharmaceutical levels and oxylipin levels were compared with those from tap water control exposure.

2. Materials and methods

2.1. Collection

Northern damselfly larvae (larval stage L-2 and L-1, second to the last and last instar before emergence, respectively) were collected in October 2019 at a pond unaffected by anthropogenic pollutants in Umeå, Sweden (63°48′03.7″N 20°18′21.8″E). Larvae were transferred to 1000 mL prewashed high density polyethylene bottles containing lake water and plant stems and brought to an aquatic laboratory at Umeå University. Here, they were fed ad libitum with zooplankton (*Daphnia pulex*) cultivated at Umeå University and kept for two days before they were further transported to Lundåkra WWTP.

2.2. Wastewater treatment plant and ozone pilot plant

In Lundåkra WWTP in Landskrona, Sweden, with a total load

corresponding to 40 000 person equivalents (PE), incoming wastewater with an annual average flow of 13 000 m^3/day is treated mechanically through screening, grit removal and sedimentation. During the experimental period, most of the mechanically treated wastewater (> 95%) was directed to an activated sludge process with nitrification, denitrification and enhanced biological phosphorous removal in a biodenipho configuration with a suspended solids concentration of 2.7 g/L and an average hydraulic retention time (HRT) of 13 h. The remaining part of the mechanically treated water was directed to a nitrifying trickling filter. Post-polishing of the biological treated water was performed through lamella sedimentation with the possibility of post-precipitation of phosphorous (not used during the experimental period).

Part of the effluent wastewater (60 m^3/d) was treated in an ozone pilot plant with a static mixer for ozone injection, an HRT of 11 min in the reaction tank and an ozone dose of 4.9 ± 0.1 g O$_3$/m^3. Further details on the ozone pilot plant are provided elsewhere (Edefell et al., 2021).

2.3. Exposure set up

Exposure was carried out within the WWTP facility according to the design in Fig. 1. Three 200 L polypropylene barrels were filled daily with water from the WWTP effluent pipeline, the post-ozonation pipeline, and non-chlorine aerated water from the drinking water network (tap water). Each barrel continuously supplied water to one exposure unit at a flow rate of 0.7 L/h. Each exposure unit (30 × 30 cm) consisted of three aquaria with separate inlets. Each aquarium was divided into eight compartments separated by a fine mesh fence (5 × 5 cm). After acclimation on-site in lake water for 24 h to allow for adjustments in temperature etc., larvae were introduced to individual compartments within the aquaria and subsequently exposed to WWTP effluents for six days. The exposure design resulted in the following exposure groups: i) conventional WWTP effluent (E, $n = 24$), ii) effluent additionally treated with ozone (O, $n = 24$), and iii) tap water control (T, $n = 24$). No food was provided during the exposure period in order to control for variability otherwise introduced by feeding. Water quality parameters (pH, temperature, dissolved organic carbon [DOC], ammonium, nitrate, nitrite) were measured daily (Table SI 1). Performance of the ozonation plant was checked throughout the exposure time (Table SI2). Additionally, water samples were collected daily and stored at −20 °C for pharmaceutical analysis. Exposure took place from Oct 24th to Oct 30th, 2019. After exposure, larvae were immediately rinsed with MilliQ water, frozen, and kept at −20 °C until extraction. Afterwards, larvae were weighed to the nearest 0.1 mg before analysis. In addition, two types of controls, naïve larvae frozen directly after collection (C$_A$, $n =$ 10, baseline control) and larvae frozen directly before exposure (C$_B$, $n =$ 10, transport and acclimation control) were analysed together with the study samples to investigate if the study conditions (handling during transport etc.) masked the response to pollutants in the water.

2.4. Pharmaceutical analysis

A total of 99 pharmaceuticals of various therapeutic classes were analysed in the water samples (conventional WWTP effluent, ozonated effluent, and tap water, n = 6, respectively) by online solid phase extraction LC-MS/MS as previously described (Lindberg, 2014). These 99 pharmaceuticals were selected based on the potential to bioaccumulate, their pharmacological potencies and sales in the region. In short, water samples were filtered and acidified, then online extraction was performed using an OASIS HLB column (20mm × 2.1 mm i.d., 15 µm particle size, Waters, Milford, Massachusetts, USA). Chromatographic separation was achieved using a Hypersil GOLD aQ C18 column (50mm × 2.1 mm i.d., 5 µm particle size, Thermo Fisher Scientific, San Jose, CA, USA). Pharmaceuticals were analysed using a TSQ Quantum Ultra EMR triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) and quantified by means of internal standards. Removal efficiencies (REs) of the identified pharmaceuticals in the ozonation step were calculated according to Equation (1):

$$RE_i = [1 - (Coz \ / \ Ceff)]x \ 100 \tag{1}$$

Where: RE_i is the removal efficiency for compound i, Coz the concentration in ozonated effluent, and Ceff the concentration in conventional WWTP effluent. If the concentration of a pharmaceutical was below its limit of quantification (LOQ), it was given the value LOQ/2.

2.5. Oxylipin analysis

Oxylipins were extracted and analysed in individual whole-body (0.0034 – 0.062 g) damselfly larvae using a previously validated LC-MS/MS method for quantification of 66 individual analytes (Späth et al., 2021). In short, larvae were placed individually in 2 mL microcentrifuge tubes and 1.5 mL acetonitrile/water were added to the larvae. Samples were homogenised using stainless steel beads and shaking for 3 min at 30 Hz in a mixer mill (MM400, Retsch Technology, Haan, Germany). Samples were centrifuged for 10 min at 14 000 RPM, supernatants were withdrawn, and samples re-extracted with 1.5 mL 90/10 acetonitrile/water, following the procedure described above. After combining of supernatants, a 1.5 mL aliquot was transferred to falcon tubes and evaporated under vacuum (MiniVac system, Farmingdale, NY,
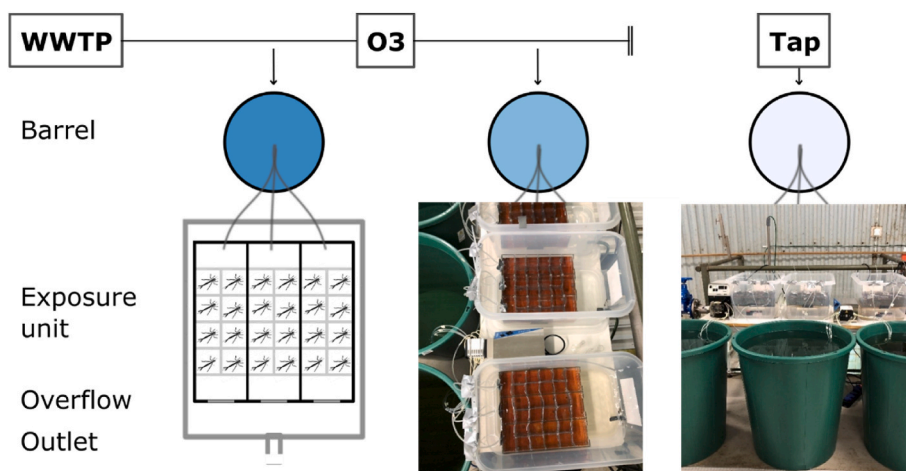

Fig. 1. Exposure set up. Damselfly larvae (n (exposure unit) = 24, N (total) = 72) were exposed to a continuous flow of either conventional WWTP effluent, effluent additionally treated with ozone, or tap water, respectively.

USA). Residues were reconstituted in 110 μL methanol and transferred to LC vials. Oxylipins were analysed using an Agilent UHPLC system Infinity 1290 (Agilent Technologies, CA, USA) coupled to an Agilent 6495 Triple Quadrupole system. Chromatographic separation was achieved using a Waters BEH C18 column (2.1 mm × 150 mm, 2.5 μm particle size). Oxylipins were quantified using internal standard based calibration curves and normalised to the wet weight of larvae (presented as ng/g).

2.6. Statistical analysis

Since data was not normally distributed, generalised linear mixed models with a gamma distribution and log-link function using aquarium as random effect and exposure type as fixed factor were carried out using IBM SPSS Statistics (version 26) to investigate how exposure affected oxylipin levels. Generalised linear models were used to determine differences in oxylipin levels between the three different control groups (C_A, C_B, T). Tukey's post-hoc test was used for multiple pairwise comparisons and determination of significantly different group means (p ≤ 0.05). To compensate for multiple comparisons, sequential Bonferroni correction was performed.

3. Results and discussion

3.1. Pharmaceutical analysis and ozone removal efficiency

Results from the pharmaceutical analysis of conventional WWTP effluent and ozonated effluent samples from Lundåkra WWTP are summarised in Fig. 2 and Table SI 3. 38 of 99 pharmaceuticals probed for were quantified in the samples. Average levels ranged from 2.6 to 4800 ng/L in conventional WWTP effluent and 2.4 to 2500 ng/L in ozonated effluent, respectively. Except for three pharmaceuticals (dipyridamole, irbesartan, losartan) at low levels (1–9% of levels found in conventional WWTP effluent) and in few of the samples, no pharmaceuticals were detected in the tap water samples. Ozonation removal efficiency was 67% on average at an ozone dose of 0.49 ± 0.04 g O3/g DOC, ranging from −21% (fluoxetine) to 99% (diclofenac). Negative removal can be caused by sorption and desorption since the particle composition in the sewage sludge is also impacted by the ozonation (Costa et al., 2022). Of the 38 detected compounds in the conventional

WWTP effluent, 16 were removed to levels below the limit of quantification (LOQ) in the ozonated effluent. Usually, removal rates of around 80% are desired, and the obtained removal is thus somewhat lower than the target. Higher removal efficiencies are achieved by higher ozone doses, but that in turn can have other disadvantages such as (more) formation of unknown or unwanted by-products and reactive species (e. g., aldehydes, ketones, carboxylic acids, bromate, see Lim et al. (2022)), higher costs, etc. So, there is a trade-off between unwanted effects and satisfactory removal rates. On-site monitoring systems to investigate the quality of effluents in terms of their impact on biota have been employed in the context of ozonation to a limited extent. However, biomarker responses in rainbow trout were investigated by Beijer et al. (2017), where CYP transcript concentrations depended on wastewater treatment strategy, including ozonation. Furthermore, conventional and ozonated WWTP effluents affected behavioural traits and reproduction in zebrafish *Danio rerio* (Pohl et al., 2018).

3.2. Effects on oxylipin levels

A total of 52 out of 66 targeted oxylipins were quantified in the damselfly larvae. Average levels ranged from 0.2 (13(14)-EpDPE) to 450 (9-oxo-ODE) ng per g (wet weight) (Table SI 4). Oxylipins derived via CYP and LOX enzymatic pathways from five different PUFAs (arachidonic acid [AA], α-linolenic acid [ALA], docosahexaenoic acid [DHA], eicosapentaenoic acid [EPA], and linoleic acid [LA]) were detected.

Two oxylipins, the fatty acid epoxides 12(13)-EpODE and 15(16)-EpODE, were significantly reduced in larvae exposed to conventional WWTP effluent in comparison to the tap water control (p < 0.05, respectively; Fig. 3; see Table SI 5 for an overview of the statistical results). Suppressed levels of both expoxides have previously been observed in larvae exposed to convential effluent compared to tap water (Späth et al., 2021). Interestingly, larvae exposed to effluent that also had been treated with ozone did not show any reduction of 12 (13)-EpODE but the reduction of 15(16)-EpODE still remained. Fatty acid epoxides are formed by CYP epoxygenase enzymes and have been previously found to be affected in mice treated with the NSAID ibuprofen (Tiwari et al., 2021). NSAIDs are commonly present in WWTP effluents, e.g., diclofenac was measured in the effluent of this study, and thus could potentially cause the observed reduction of fatty acid epoxides in damselfly larvae exposed to WWTP effluents, however little is
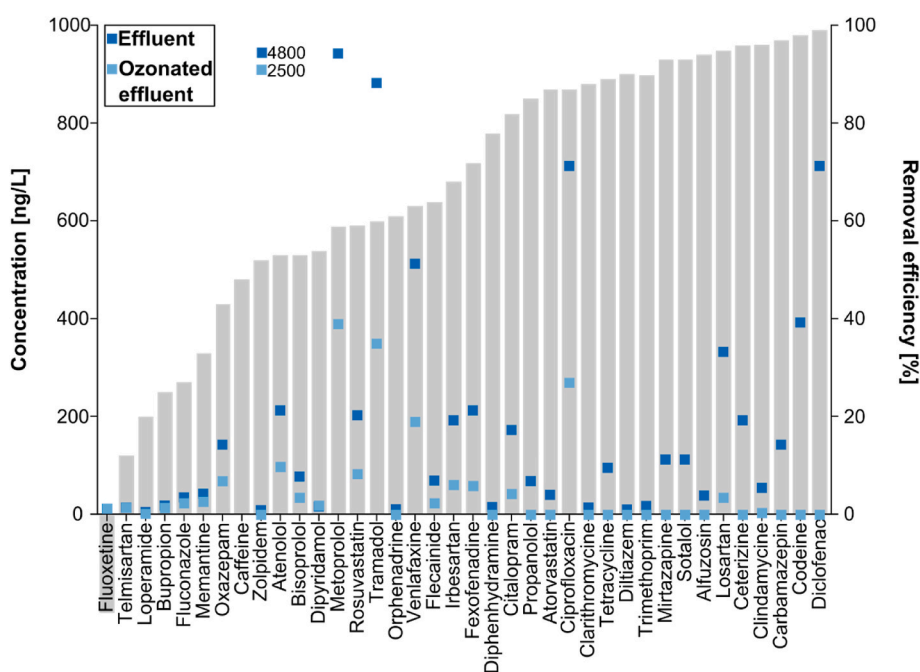

Fig. 2. Average concentrations (blue squares) and removal (grey bars) of quantified pharmaceuticals in conventional WWTP effluent (n = 6; dark blue) and after additional treatment by ozonation (n = 6; bright blue) in Landskrona WWTP. Removal efficiency (%) of the ozonation step was calculated based on average concentrations of pharmaceuticals in conventional WWTP effluent and ozonated WWTP effluent. If the concentration of a pharmaceutical was below its limit of quantification (LOQ), it was given the value LOQ/ 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
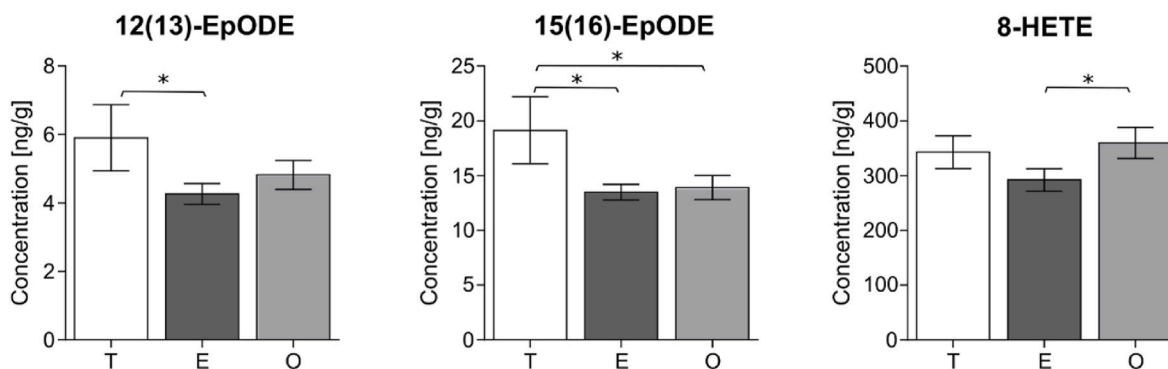
Fig. 3. Oxylipin concentrations (ng/g) in damselfly larvae exposed to tap water (T), conventional WWTP effluent (E), effluent additionally treated with ozone (O) (*p < 0.05; corrected using Bonferroni).

known on the biochemical mechanism behind this response in general, and especially in invertebrate species. One oxylipin, 8-HETE, was not significantly different from exposure to the tap water control, but was significantly lower in larvae exposed to conventional WWTP effluent compared to larvae exposed to ozonated effluent (Fig. 3). Levels of 8-HETE were previously shown to be significantly affected in damselfly larvae after a longer exposure period (16 days) to conventional WWTP effluent (Späth et al., 2021). In contrast to our lab-scale pilot study with comparable exposure durations (Späth et al., 2020), we found fewer effects of effluent exposure and, overall, affected oxylipins were not in full agreement with previously responsive ones.

There are at least three potential explanations to the observed variations, in terms of responsive oxylipins, among the studies. First, these differences could be due to different composition of the effluents and/or the result of the respective ozonation steps. The effluent in the previous experiment (Späth et al., 2020) was obtained at a larger WWTP (166 000 PE, annual average flow of 30 000 m3/day) without nitrogen removal, and contained fewer, but potentially more potent, pharmaceuticals (25 compared to 38 in this study, of which 22 were detected in both effluents) at lower average and total concentrations (analysed with the same method as described above, data not shown). Ozonation of the WWTP effluent was performed in a lab scale column reactor at a slightly higher ozone dose of 5.5 g O_3/m3 resulting in a higher average removal efficiency of 80% (data not shown) compared to 67% in this study. We compared the pharmaceuticals measured in effluents of both studies and found that the antidepressants amytriptyline, mianserin, and sertraline, which were only detected in the effluent of the former study, were all subsequently removed to levels below their LOQ by the ozone treatment. To test whether the differences in observed oxylipin changes across the two studies could be caused by any of these three pharmaceuticals, single exposure experiments should be carried out in future studies.

Second, damselfly larvae were collected in different years and seasons (August 2017 vs October 2019). Resulting differences in temperature, light, food availability etc., could cause differences in oxylipin baseline levels. No naïve individuals, i.e., larvae directly frozen after collection, were analysed in previous studies to compare baseline levels, which should be included in future studies to investigate seasonal variation and to enhance comparability across different studies.

Third, the differences could be caused by the exposure set up. Earlier studies were carried out in a quiet lab environment, using larger opaque aquaria (10 × 10 × 10 cm), and having no exchange of the exposure medium, whereas the present study was done in a more noisy environment at the treatment plant facility, using mesh separated and smaller compartments (5 × 5 × 5 cm), and a flow-through system. The conditions in this study could potentially have stressed the study individuals, which in turn could have masked the physiological stress caused by pollutants in the water. To test this assumption, using generalised linear models, we compared oxylipins of naïve individuals (C_A), i.e., larvae that were frozen directly after collection to the transport, with

acclimation control (C_B), i.e., larvae that were frozen directly before exposure, and tap water control (T), i.e., larvae that were exposed to tap water in the treatment facility. We found that both transport/acclimation and exposure itself resulted in a number of significant oxylipin changes (12 in C_A vs C_B, 11 in C_B vs T, respectively; Table SI 6).

The experimental set-up might have affected oxylipin levels by inducing a stress response that was different from previous lab-scale experiments. Hence, great caution has to be taken in designing exposure experiments for detecting sub-lethal effects (or more specifically oxylipin levels responsive to ozonation) in exposed biota. It is likely that other organisms are sensitive to handling and variations in experimental setup, so future studies should consider the most robust sentinel species in terms of the sub-lethal effect under study and include organisms beyond damselfly larvae using an expanded panel of endpoints. Furthermore, control larvae were exposed to tap water instead of lake water to eliminate effects caused by the potential presence of pollutants in the lake water. Since tap water differs, in many aspects e.g., chemical properties, from the lake water that damselfly larvae naturally inhabit, exposure to tap water could have contributed to the differences in oxylipin levels of transport and acclimation (using lake water) and the exposed tap water control. Future studies are necessary to address the effect that exposure to tap water might have on oxylipin levels compared to lake water exposure. Additionally, these differences between controls could be explained by the change/lack of diet, since the larvae were not fed during transport, acclimation, and exposure (increased time without food) and oxylipin levels may vary based on diet in damselfly larvae in line with dietary effects in humans (Nording et al., 2013; Schuchardt et al., 2014; Gouveia-Figueira et al., 2015).

Overall, our results highlight the importance of using suitable controls and characterising metabolite baseline levels when assessing risks posed by anthropogenic pollutants.

Based on the assumption that oxylipin responsiveness could be masked by the study design, for exploratory purposes oxylipins that were affected by exposure at the more liberal significance level of p < 0.1 were identified. Similar trends as above were found; levels of five oxylipins including three fatty acid epoxides (9(10)-EpODE, 8(9)-EpETE, 17(18)-EpETE), as well as 12-HETE, and 5-HEPE were reduced in larvae exposed to conventional WWTP effluent in comparison to larvae exposed to tap water (Fig. SI 1). In contrast to conventional WWTP effluent, exposure to ozonated effluent did not affect levels of three oxylipins (9(10)-EpODE, 12-HETE, and 8(9)-EpETE), while it led to reduced levels of three oxylipins (17(18)-EpETE 5-HEPE 12(12)-EpETE). Furthermore, 8,9-DiHETrE was reduced in larvae exposed to conventional WWTP effluent compared to larvae exposed to ozonated effluent. We conclude that in particular CYP-derived fatty acid epoxides, such as EpODEs (via ALA) and EpETEs (via EPA), may be relevant endpoints when assessing sub-lethal effects.

Additional limitations of the study include uncontrolled light conditions and although flow-through, the exposure set up was not flow-

proportional and thus it did not reflect alterations of effluent compositions during a 24-h period. Furthermore, damselfly larvae were only exposed for 6 days, whereas in nature they are exposed for the majority (if not all) of their larval life. Exposure experiments over an organism's entire aquatic ontogeny is rare, especially in animals with relatively long (years) generation time, but should be encouraged as it increases the ecological relevance and with that the precision of the risk-assessment (e.g., Thoré et al. (2021)). Addressing these limitations requires further studies on larvae baseline metabolism and a stringent experimental design to facilitate on-site assessment of WWTP effluent exposure.

4. Conclusion

Ozonation of conventional WWTP effluent resulted in an average removal efficiency of 67% in measured pharmaceuticals. Two oxylipins in damselfly larvae were responsive to conventional WWTP effluent exposure, of which one was also responsive to ozonated effluent exposure compared to the tap water control. One oxylipin was significantly lower in larvae exposed to conventional WWTP effluent compared to larvae exposed to ozonated effluent. The proposed experimental set-up showed promise in assessing sub-lethal effects of effluent exposure on-site the WWTP. However, modifications of the study design with regard to an even more controlled set-up are required to successfully implement the method for evaluating wastewater treatment techniques, such as ozonation, in terms of potential sub-lethal effects on the oxylipidome.

Supporting information

- Additional experimental details and other experimental results

Author statements

Jana Späth: Conceptualization, Project administration, Investigation, Writing- Original draft preparation, Writing – review & editing. Tomas Brodin: Conceptualization, Formal analysis, Supervision. Per Falås: Investigation. Mirva Niinipuu: Investigation. Richard Lindberg: Investigation. Jerker Fick: Conceptualization, Supervision. Malin Nording: Supervision, Funding acquisition, Writing- Original draft preparation, Writing – review & editing.

Data statement

The dataset used during the current study is available from the corresponding author on reasonable request.

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.

Data availability

Data will be made available on request.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.136604.

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