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A burning issue: The effect of organic ultraviolet filter exposure on the behaviour and physiology of *Daphnia magna*

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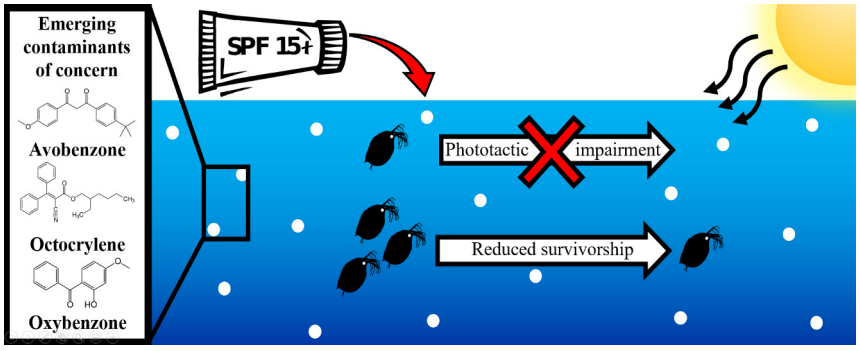
HIGHLIGHTS

- *D. magna* neonates were exposed to ultraviolet filters (UVFs) for 2 or 21 days.
- 48-hour UVF exposure reduced survivorship and behaviourally impaired daphnids.
- Chronic exposure to 7.5 μg/L octocrylene resulted in complete 7-day mortality.
- Chronic exposure to avobenzone or oxybenzone disrupted daphnid metabolic rate.
- UVFs exhibit toxicity to *Daphnia* at environmentally realistic concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Ultraviolet (UV) filters are compounds utilized in many manufacturing processes and personal care products such as sunscreen to protect against UV-radiation. These highly lipophilic compounds are emerging contaminants of concern in aquatic environments due to their previously observed potential to bioaccumulate and exert toxic effects in marine ecosystems. Currently, research into the toxic effects of UV filter contamination of freshwater ecosystems is lacking, thus the present study sought to model the effects of acute and chronic developmental exposures to UV filters avobenzone, oxybenzone and octocrylene as well as a mixture of these substances in the freshwater invertebrate, *Daphnia magna*, at environmentally realistic concentrations. Median 48-hour effect and lethal concentrations were determined to be in the low mg/L range, with the exception of octocrylene causing 50% immobilization near environmental concentrations. 48-hour acute developmental exposures proved to behaviourally impair daphnid phototactic response; however, recovery was observed following a 19-day post-exposure period. Although no physiological disruptions were detected in acutely exposed daphnids, delayed mortality was observed up to seven days post-exposure at 200 μg/L of avobenzone and octocrylene. 21-day chronic exposure to 7.5 μg/L octocrylene yielded complete mortality within 7 days, while sublethal chronic exposure to avobenzone increased *Daphnia* reproductive output and decreased metabolic rate. 2 μg/L oxybenzone induced a 25% increase in metabolic rate of adult daphnids, and otherwise caused no toxic effects at this dose. These data indicate that UV filters can exert toxic effects in freshwater invertebrates, therefore further study is required. It is clear that the most well-studied UV filter, oxybenzone, may not be the

Abbreviations: AVO, avobenzone; FDA, Food and Drug Administration (US); EC_{50}, median effect concentration; LC_{50}, median lethal concentration; OCT, octocrylene; OXY, oxybenzone; OECD, Organization for Economic Co-operation and Development; WWTP, wastewater treatment plant; UVF, ultraviolet filter.

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most toxic to *Daphnia*, as both avobenzone and octocrylene induced behavioural and physiological disruption at environmentally realistic concentrations.

1. Introduction

Organic ultraviolet filters (UVFs) such as oxybenzone, avobenzone and octocrylene are emerging as contaminants of concern in aquatic environments (Downs et al., 2016). These compounds are utilized in hundreds of pharmaceutical and personal care products, often in tandem due to their properties of synergistic stabilization of other UVFs, and serve primarily as the active ingredients in sunscreen to protect skin against damage from UV-A and UV-B radiation (Berardesca et al., 2019; Committee for Human Medicinal Products, 2015; Gomez et al., 2012; Manová et al., 2013; Vione et al., 2013). There are two primary modes of entry for UVFs into the aquatic environment; 1) aquatic recreational activity promoting the leaching of UVFs from the skin (Labille et al., 2020; Sánchez Rodríguez et al., 2015; Schaap and Slijkerman, 2018), and 2) point sources such as wastewater treatment plant (WWTP) effluent due to the incomplete removal of these substances from manufacturing process water (Gago-Ferrero et al., 2013b; Semones et al., 2017; Wang and Wang, 2016). As a result, the measured concentrations near beaches fluctuate with recreational activity, peaking at 7.3 μg/L in coastal areas (Bargar et al., 2015; Horricks et al., 2019; Langford and Thomas, 2008; Sánchez Rodríguez et al., 2015), 10–44 μg/L in rivers, and reaching upwards of 200 μg/L in WWTP effluent (Kasprzyk-Hordern et al., 2009).

Recent studies have suggested that UVFs are potentially toxic in many different species, warranting further study into identifying their mechanisms of action. Oxybenzone, octocrylene and avobenzone have all been found to systematically absorb into human blood plasma at concentrations up to 210 ng/mL, 8 ng/mL and 4 ng/mL respectively (Matta et al., 2019). These concentrations exceed the current US Food and Drug Administration (FDA) guidelines for toxicity testing of 0.5 ng/mL for unknown toxicants, ultimately leading to an FDA request for further research into these active ingredients (Committee for Human Medicinal Products, 2015; US Food and Drug Administration (FDA), 2016). Indeed, oxybenzone is a known cause of photobleaching in corals at concentrations as low as 2 μg/L (Downs et al., 2016), and induces reproductive inhibition in marine algae (Lee et al., 2020; Rodil et al., 2009). Octocrylene effect concentrations for several marine invertebrate species fall within the range of measured environmental concentrations (see above) (Giraldo et al., 2017). Currently, the potential mechanism of action for these UVFs is thought to be endocrine disruption, exhibiting anti-androgen-like activities and disrupting estrogen receptor signalling (Downs et al., 2016; Klopčič and Dolenc, 2017). However, our current knowledge of the toxicity of these UVFs in freshwater and marine environments is currently insufficient, and with the given high lipophilicity of these compounds the potential risk of bioaccumulation is high (Cocci et al., 2020; Gago-Ferrero et al., 2013a; He et al., 2019; Horricks et al., 2019; Lee et al., 2020; Martín et al., 2020). Environmental contamination poses a potential threat to aquatic organisms, as little time has passed to allow for evolutionary adaptation to occur for these novel toxicants (Tierney and Kennedy, 2008). It is clear that further studies in freshwater environments are required in an effort to comprehensively understand the potential effects of UVF contamination.

Daphnia magna are a promising candidate organism to model the effects of environmental UVF exposure in freshwater invertebrates. The well-documented sensitivity of daphnids to environmental stressors, particularly through reproductive and migratory behaviours, allow for the identification of subtle, yet ecologically relevant changes in addition to more traditional physiological endpoints such as mortality and direct measurements of metabolic processes (Altshuler et al., 2011). Additionally, *Daphnia* serve as an important transitional species in the food web (Hartnett, 2019), linking primary producers to higher trophic levels that may be more susceptible to UVF-mediated environmental disturbances. This includes biomagnification through zooplankton bioaccumulation (Shi et al., 2020; Wang et al., 2019) or a lack of prey species availability as a result of population collapse due to reproductive inhibition or overt mortality (Wathne et al., 2020).

Overall, this study aimed to combine physiological and ecological endpoints in an effort to understand the environmental relevance of the UVFs avobenzone, octocrylene and oxybenzone in a keystone invertebrate species (*D. magna*). Our objectives were to:

1) Determine the concentration necessary for severe physiological impairment in acute exposures.
2) Assess the extent to which behavioural and physiological recovery is possible at environmentally relevant concentrations.
3) Assess the effects of chronic UVF exposure throughout the *D. magna* lifespan.

By combining acute and chronic developmental exposures, a more complete assessment of the risks of UV-filters to freshwater invertebrates can be made. These data are crucial to inform policy regarding the use of these compounds, particularly as several jurisdictions consider implementing restrictions or outright bans of several UVFs (Schneider and Lim, 2019).

2. Materials and methods

2.1. Daphnia colony maintenance

Daphnia magna obtained from Aquatic Research Organisms (USA, September 2019) were used to culture a colony at the University of Alberta Biological Sciences department. *Daphnia* were housed in 1 L of dechlorinated City of Edmonton water (pH ≈ 7.6) in 2 L glass beakers, prepared according to Organization for Economic Co-operation and Development (OECD) guidelines (294 mg/L $CaCl_2$, 123 mg/L $MgSO_4$, 64.8 mg/L $NaHCO_3$, 5.80 mg/L KCl) (OECD, 2008). The complete water chemistry of dechlorinated City of Edmonton water has been previously described in Delompré et al. (2019b). Colonies were maintained at 20 ± 1 °C on a 12 h light:12 h dark photoperiod and fed 5 mL once daily of both freshwater green algae (FKA *Selenastrum capricornutum*) and YCT Mix (yeast, cereal leaf, trout chow) supplied by Aquatic Research Organisms (USA). The diets were supplemented once weekly with 100 μL of Roti-Rich invertebrate food (VWR, Edmonton, Alberta, Canada). 100% water changes were performed every 2–3 days.

2.2. Exposure solutions

UV filters oxybenzone (OXY), avobenzone (AVO) and octocrylene (OCT) were dissolved in dimethyl sulfoxide (DMSO) to produce working stock solutions (MilliporeSigma, Canada; Table I). These stocks were further diluted into OECD water for treatment exposures. Treatment groups consisted of individual UV filters, or a 1:1:1 mixture of AVO, OXY and OCT. Mixture concentrations are labelled throughout as the concentration of any individual UV filter component (ex. 200 μg/L each of AVO, OCT and OXY is listed as 200 μg/L mixture). Two controls were run, the first as OECD water without additions, the second as 1.0 μL/mL (0.1% *v*/v) DMSO, an equivalent concentration to the solvent used in all treatments as a vehicle control. Prior to exposures, all

Table I
Summary of stock solution parameters measured by ultra-performance liquid chromatography.

Stock	Nominal concentration (g/L)	Measured concentration (g/L)	Detection limits (μg/L)
AVO	10	10.00 ± 0.1	50
OCT	100	100.0 ± 0.5	100
OXY	10	10.00 ± 0.1	1.0
DMSO control	0	AVO: bdl OCT: bdl OXY: bdl	

bdl: below detection limit.

glassware was submerged in 10% EtOH for >12 h then rinsed with distilled water.

2.3. Analytical methods

The stock solutions previously described in section 2.2 were analyzed via ultra-performance liquid chromatography – quadrupole time-of-flight mass spectrometry (Xevo G2-S, Waters) and the mass range of 200–300 (*m/z*). The electrospray ionization source was operated in positive ion mode. Chromatographic separation was achieved using ACQUITY UPLC BEH C18, 50 × 2.1 mm column, at 40 °C with an injection volume of 10 μL. The mobile phase consisted of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B), and the flow rate was set at 0.4 mL/min. The elution gradient was at 0–5 min, solvent B was increased from 30% to 50%, 5–8 min solvent B was increased to 95% and held at 95% for 1.5 min. Data acquisition was controlled using MassLynx (Waters) and data extraction was performed using TargetLynx (Waters). The criteria for the limit of detection is 3 times the signal to noise ratio. The detection limit of each compound was determined through a series of dilutions to determine the lowest concentration yielding a signal to noise ratio of 3.

2.4. EC_{50} and LC_{50}

OECD guidelines were followed for a determination of acute 48-hour median effect concentrations (EC_{50}), as well as lethal concentration (LC_{50}) in <24 h old neonates. The chosen effect for EC_{50} determination was immobilization, defined as an inability of a neonate to move after a gentle agitation over an observation period of 15 s (OECD, 2004). Daphnids were viewed under a dissecting microscope to differentiate mortality and immobilization as the cause of a lack of mobility by observing the presence of gill motion during respiration. 5 neonates housed in 10 mL of each solution and fasted for the entire duration of the experiment were used for each concentration tested, replicated 6 times.

2.5. Acute exposures

21-day reproductive tests were performed on daphnids following OECD test 211 guidelines, with modifications (OECD, 2008). < 24 h old neonates were exposed for 48-hours in treatment solutions prior to being raised an additional 19 days in OECD water to an age of 21 days total. Treatment concentrations were 0.2, 2, 20, and 200 μg/L of AVO, OCT, OXY or the UVF mixture. 30 neonates were exposed to each UVF treatment and housed individually in 20 mL glass scintillation vials. In addition, 15 daphnids were each exposed to DMSO and OECD control solutions alongside each concentration of UVF exposure. All exposure solutions were refreshed every 2 days for the duration of the test and *Daphnia* were fed 100 μL of both green algae and YCT mix daily unless otherwise specified. Each daphnid was checked daily for mortality, molting and any release of broods. At the end of the 2-day acute exposure but prior to transferring neonates to vials of fresh OECD water, a phototaxis assay was performed, outlined in Delompré et al. (2019a). Briefly, neonates were transferred to the centre of a 34 cm long, 3 cm diameter clear polycarbonate tube containing 175 mL of fresh OECD water, housed inside of a closed, opaque dark box. The outer 5 cm of one end of each tube protruded from the container above an illuminated lightbox (approximately 170 lm) in an otherwise dark room, and the time elapsed for daphnids to move into the illuminated tube section was recorded. Daphnids were allowed a maximum of 5 min from the moment the tubes were illuminated to complete the assay, otherwise the trial was considered incomplete. This phototaxis assay was repeated on the same neonates at the end of experimental day 21.

2.6. Chronic exposures

Chronic exposures were performed following the procedures described in section 2.5, with modifications. A first group consisted of UVF exposures at approximately 10% of the estimated EC_{50} concentration (AVO = 150 μg/L, OCT = 7.5 μg/L, OXY = 100 μg/L), followed by a second exposure group at concentrations approximately an order of magnitude lower (AVO = 20 μg/L, OCT = 0.5 μg/L, OXY = 2 μg/L). Both groups were exposed alongside OECD control solutions. Due to a lack of unique results in both of the previous experiments, mixture exposures were not included in the chronic exposures. Daphnids were fasted for the final 48 h of exposure, prior to measuring metabolic rate at the end of the 21-day experimental period. Metabolic rate was measured by pairing 2 daphnids per 200 μL well filled with aerated OECD water in a 24-well glass optical fluorescence respirometry microplate (Loligo Systems, Denmark). The dissolved oxygen concentration ($mgO_2\ L^{-1}$) was logged over 1.5 h, and the per daphnid respiration rate was calculated by dividing the slope (change in oxygen concentration over time, in hours) by the number of daphnids per well (2).

2.7. Statistics

All statistical analyses were performed using R version 3.6.2 (R Core Team, 2019). EC_{50} and LC_{50} determination was performed using the "ecotox" package. Tests of normality and homoscedasticity (Shapiro-Wilk and Levene's tests, respectively) were performed on all data prior to testing via one-way ANOVA. Data that failed assumption tests and could not be successfully transformed were compared by Kruskal-Wallis test followed by post-hoc Dunn's test. Significance was determined at $\alpha = 0.05$. Values are reported as mean ± standard error of the mean.

3. Results

3.1. Exposure solution chemistry

A summary of nominal and measured concentrations of AVO, OCT and OXY stock solutions as well as the detection limits of each compound are included in Table I. All measured UVF concentrations were consistent with their respective target nominal concentrations (AVO, OXY: 10 ± 0.1 g/L, OCT: 100 ± 0.5 g/L, DMSO control: all UVFs below detectable limits).

3.2. EC_{50} and LC_{50}

The EC_{50} concentration was found to be far lower with OCT at 0.03 mg/L (95% CI 0.02–0.04) than with either OXY (EC_{50} = 1.2 mg/L; 95% CI 0.89–1.6) or AVO (EC_{50} = 1.2 mg/L; 95% CI 0.91–1.6; Fig. 1A-C).

All LC_{50}'s were determined to be within 1 order of magnitude in the low mg/L range, the lowest of which was the 1:1:1 UVF mixture at 0.99 mg/L (95% CI 0.82–1.2), followed by OXY (1.7 mg/L; 95% CI 0.74–5.0), OCT (3.6 mg/L; 95% CI 2.5–5.2), and AVO at 6.8 mg/L (95% CI 4.8–9.4; Fig. 1D-G). The calculated LC_{50} for the DMSO control was 2.3% *v*/v in OECD water (95% CI 1.5–4.2), and the no effect concentration

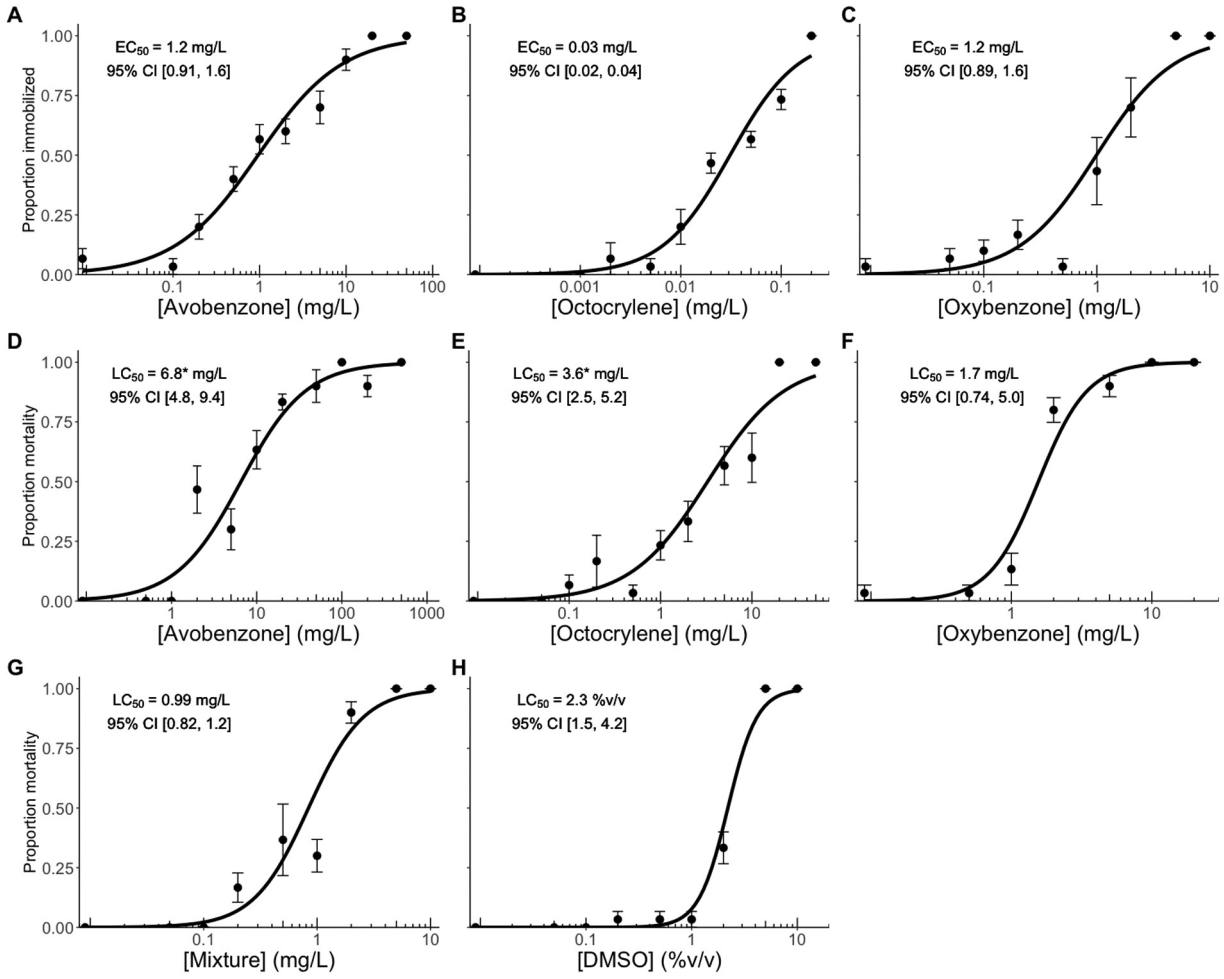

Fig. 1. Median effect concentrations causing immobilization (EC_{50}) (A-C) and median lethal concentrations (LC_{50}) (D-G) of <24-hour old *Daphnia magna* neonates exposed to individual UV filters and a 1:1:1 mixture for 48 h. The LC_{50} of the vehicle control, dimethyl sulfoxide (DMSO) was determined to select the solvent concentration used for all UVF exposures (H). Each data point represents 6 replicates of $n = 5$ daphnids, bars represent ± SEM. *Indicates values that are above the solubility limit in water.

was 1.0% v/v, well above the solvent concentration of 0.1% v/v used for all test exposures (Fig. 1H).

3.3. Acute developmental exposures

Overall, no behavioural impairment was observed after a 48-hour exposure to UV filters (Kruskal-Wallis, $p > .07$); however, post-hoc analysis revealed that 200 μg/L of all treatments excluding OXY yielded an increased phototactic response latency, to a maximum of 87 ± 19 s in daphnids exposed to AVO, representing a 35% increase over OECD controls (Dunn's, $p < .01$; Fig. 2A). OCT demonstrated a log-linear relationship with dose, to a maximum impairment of 74 ± 15 s (Dunn's, $p < .01$). 200 μg/L UVF mixtures produced results similar to their component chemicals, increasing response time by 62 ± 19 s in comparison to the OECD control (Dunn's, $p > .10$). All differences in behavioural responses to light stimuli were absent following 19 days of recovery in OECD water (Kruskal-Wallis, $p > .10$; Fig. 2B).

Physiological effects of UVF exposure were not detected in any of the traditional endpoints measured with 21-day OECD 211 experiments. The number of neonates produced by reproductive daphnids did not vary from either OECD or DMSO controls by more than 10% in AVO and OXY treatments (Kruskal-Wallis, $p > .05$; Fig. 3A). Post hoc analysis revealed two statistically significant exceptions, as marginally more neonates were produced in 200 μg/L OCT exposures and fewer neonates in 20 μg/L mixture exposures (Kruskal-Wallis, $p < .01$). The first brood of neonates was released within ±1 day for every treatment, a minimum of 11 days to a maximum of 13 days (Fig. 3B). Molting behaviour was also unaffected by every treatment, as each daphnid molted once every 2–3 days for an average of 8.9 ± 1.1 molts (OECD control) over the duration of the experiment (Fig. 3C). It should be noted that statistical significance was detected in AVO treatments, as 200 μg/L treatments molted 9.4 ± 1.4 times (Kruskal-Wallis, $p < .01$; Fig. 3C).

The mortality rate of acutely exposed daphnids was unaffected by any treatment group at concentrations ≤20 μg/L over 21 days ($p > .70$; Fig. 4A-C). No significant mortality was observed in any treatment at 200 μg/L for the duration of the 48-hour exposure (Kruskal-Wallis, $p > .10$; Fig. 4D). However, a sharp increase in mortality rate was observed in daphnids exposed to AVO, OCT and UVF mixtures from days 3–7, with the greatest decline observed on day 4, followed by a return to normal rates on day 8 (Kruskal-Wallis, $p < .05$). Daphnid populations stabilized with a survivorship proportion of 0.63 ± 0.03 for AVO, 0.13 ± 0.07 for OCT and 0.13 ± 0.13 for mixtures over the remainder of the observation period. Acute exposure to OXY at 200 μg/L did not adversely impact daphnid survivorship in comparison to both control groups at any point over the 21-day period ($p > .40$).

3.4. Chronic developmental exposures

Chronic exposures to AVO, OCT and OXY at approximately 10% of EC_{50} concentrations did not greatly impact the mortality rate of

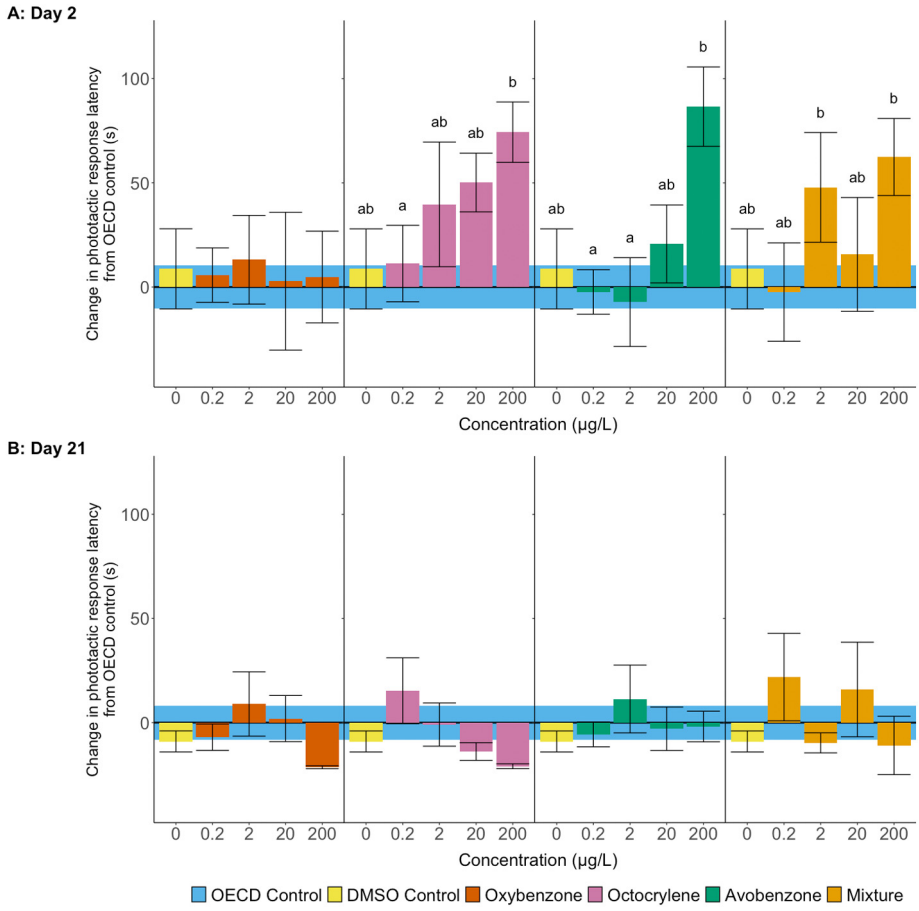

Fig. 2. The mean change in phototactic response time in comparison to OECD water controls of <24-hour old *Daphnia magna* neonates after a 48-hour exposure (A) and 19 days post exposure (B) to individual UV filters and a 1:1:1 mixture. The trial was considered as incomplete if daphnids did not finish in ≤5 min. Each treatment group was comprised of 30 individuals. Bars represent mean ± SEM. Different letters on the bars indicate a significant difference between concentrations within treatments, bars without letters are not considered to be significantly different (Kruskal-Wallis, $p < .05$).

daphnids until day 5, at which point mortality reached approximately 50% for AVO (150 µg/L) and OCT (7.5 µg/L) exposed groups (Fig. 5). 100% mortality was reached on day 7 in both cases. 100 µg/L OXY increased the mortality rate to 0.33 on day 9, and all remaining daphnids were deceased on day 10. A second batch of chronic exposures to lower concentrations of UVFs did not affect the mortality rate with respect to the OECD control group at any point over the 21-day exposure (AVO = 20 µg/L, OCT = 0.5 µg/L, OXY = 2 µg/L).

The time to produce the first brood was unaffected by all UVFs in the second, lower concentration batch of chronic exposures (Kruskal-Wallis, $p < .05$; Fig. 6A), as well as molting behaviour ($p > .20$; Fig. 6E). 20 µg/L AVO increased the average brood size per daphnid by nearly 1.5-fold over OECD controls to 13 ± 0.85 neonates/brood (Kruskal-Wallis, $p < .01$; Fig. 6B). This is similarly reflected in a 20% increase in the proportion of daphnids reproducing at any point over the 21-day exposure compared to controls (Fig. 6C). Overall, the average number of neonates produced per reproductive daphnid increased in AVO-exposed individuals by 40% (Kruskal-Wallis, $p < .01$; Fig. 6D). Metabolic rate increased by 25% (4.8 ± 0.49 mgO_2L^{-1} h^{-1}) in 0.5 µg/L OXY treated daphnids and decreased by 25% in 20 µg/L AVO treatments; however, these differences were not statistically significant (Kruskal-Wallis, $p > .10$; Fig. 6F).

4. Discussion

These results represent the first investigation into the effects of UVF exposure on the behaviour and physiology of *Daphnia magna*, as well as the persisting effects over a 21-day period following both acute and chronic exposures to AVO and OCT. Short-term, developmental exposure impaired the phototactic response of neonates towards light stimuli, in addition to inducing an increased mortality rate up to 7 days post-exposure. These physiological effects are generally recoverable over a two-week period in surviving daphnids. Chronic developmental exposures prove to be more disruptive to *Daphnia* reproduction, particularly to environmentally relevant concentrations of AVO. OCT exposure was shown to be highly lethal over a 1-week period at environmentally relevant concentrations. Interestingly, OXY, the most studied UV filter, was shown to generally be the least toxic of the studied compounds, indicating that the primary focus of research effort should be expanded to include other compounds such as AVO and OCT.

4.1. 48-hour acute toxicity

Of the studied compounds, AVO and OXY were equivalently toxic, inducing 25% immobilization at concentrations <1 mg/L, and complete immobilization at approximately 10 mg/L. OCT immobilized all daphnids at 0.2 mg/L, indicating a higher level of toxicity to developing *Daphnia* than AVO and OXY (Fig. 1A-C). The determined OCT EC_{50} is two orders of magnitude less than that determined by Park et al. (2016), at 3.2 mg/L; however, this is the only known literature value for this compound at the time of writing. The difference in observed toxicity could be a result of water chemistry differences (City of Edmonton, Canada derived OECD water vs Saarbrücken, Germany filtered recirculating tap water), or differential toxicant sensitivity across separate daphnid

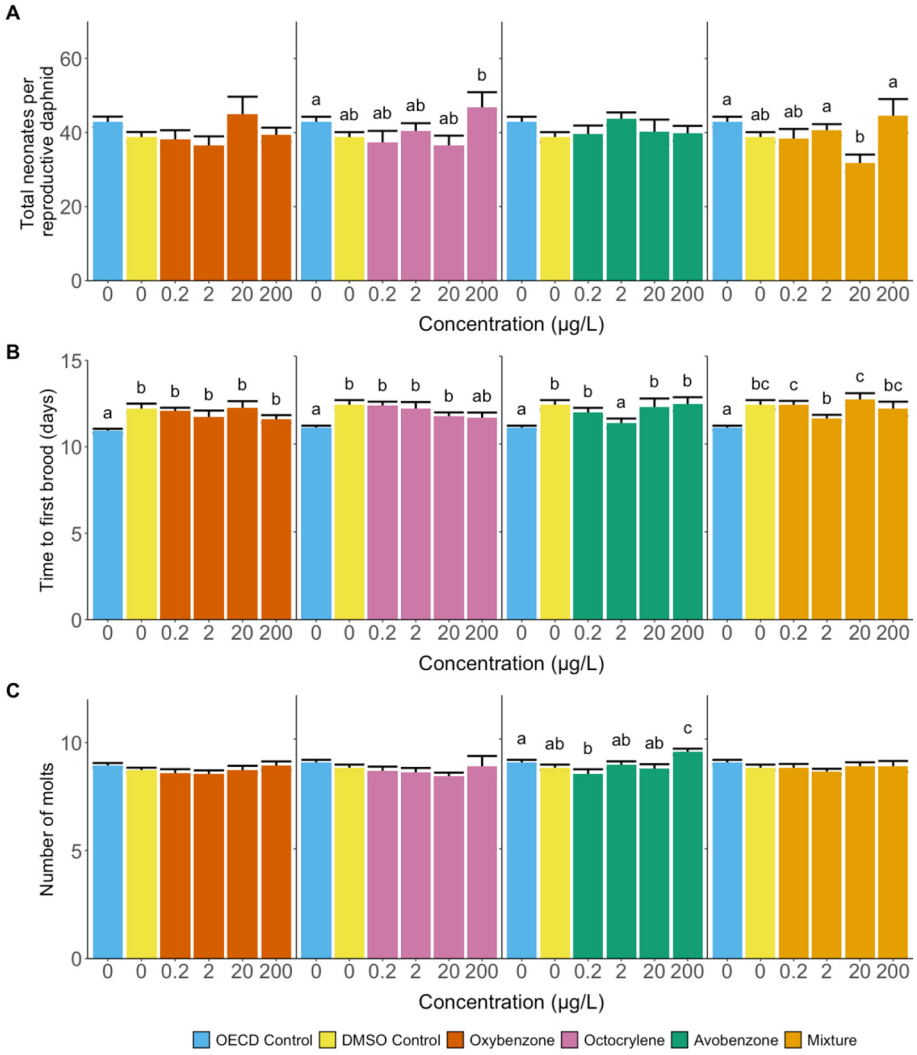

Fig. 3. The number of neonates produced over 21 days per reproductive daphnid (A), the mean time to the release of the first brood (B) and the number of molts over 21 days (C) of <24-hour old *Daphnia magna* neonates exposed to individual UV filters and a 1:1:1 mixture for 48 h. Each treatment group was comprised of 30 individuals. Bars represent mean ± SEM. Different letters on the bars indicate a significant difference between concentrations within treatments, bars without letters are not considered to be significantly different (Kruskal-Wallis, $p < .05$).

cultures (Reproductive adults sourced from Aquatic Research Organisms, USA vs ephippia sourced from MicroBioTest Inc., Belgium). The AVO EC_{50} is comparable to the value presented in this study, at 2.0 mg/L, as well as the variety of published values for OXY-exposed *Daphnia*, ranging from 1.2–2.2 mg/L (Jang et al., 2016; Molins-Delgado et al., 2016; Sieratowicz et al., 2011). The acute toxicity of OCT is noteworthy due to its proximity to previously measured environmental concentrations of approximately 7 µg/L in surface waters, as EC_{50} measurements are a more ecologically sensitive measure of toxicity, and immobility can lead to organism death over a longer period of time due to predation (Blewett et al., 2018; Bratkovics et al., 2015; Langford and Thomas, 2008; Schaap and Slijkerman, 2018).

Short-term exposure to any of the tested UVF solutions did not prove to be immediately lethal, as all LC_{50} doses were determined to be at concentrations above what has been measured in the environment. The calculated LC_{50} doses for AVO, OCT and OXY were 6.8, 3.6, and 1.7 mg/L respectively, and 0.99 mg/L for an equivalent ratio mixture of these compounds (Fig. 1D-G). These results are comparable to previously published research on OXY, ranging from 1.6–1.9 mg/L (Fent et al., 2010; Mikkelsen, 2015; Montes-Grajales et al., 2017). The 48-hour LC_{50} determined for AVO and OCT are the first to be reported in *D. magna*, and are similar to doses predicted by toxicity modelling (Brooke et al., 2008). It is important to note that due to the high lipophilicity of organic UVFs, the assessed LC_{50} doses are highly unlikely to occur under normal environmental conditions, requiring a solvent such as DMSO to maintain consistent high concentrations. The solubility of AVO and OCT in water are 2.2 and 0.36 mg/L respectively, and the maximum solubility for OXY is above the median lethal concentration, at 210 mg/L (Giraldo et al., 2017; Molins-Delgado et al., 2016; National Center for Biotechnology Information, 2020).

4.2. Physiological effects

Acute 48-hour UVF exposure did not alter *Daphnia* reproductive or molting behaviour over the post-exposure period (Fig. 3). In a previous experiment by Sieratowicz et al. (2011), the authors showed that chronic OXY exposures <0.5 mg/L did not impact the reproductive capabilities of daphnids, suggesting that very high doses are required to observe a physiological response after a 48-hour exposure. Chronic exposure to 20 µg/L AVO increased the reproductive output of daphnids by 1.4-fold, driven by a higher proportion of reproducing adults and a larger average brood size (Fig. 6B-D). *Daphnia* have been shown to increase in reproductive rate when influenced by many environmental and chemical stressors, this phenomena may be in accordance with

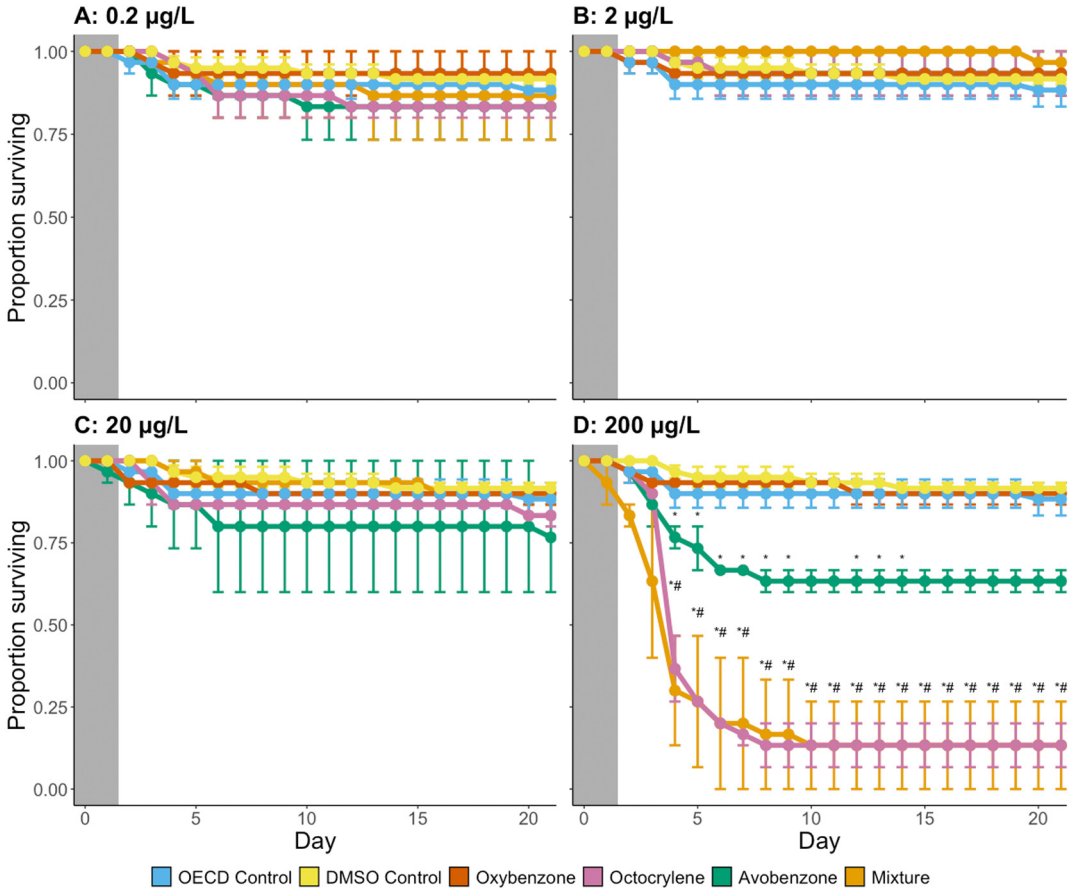

Fig. 4. Survivorship curves of <24-hour old *Daphnia magna* neonates exposed to individual UV filters and a 1:1:1 mixture for 48-hours at concentrations of 0.2 µg/L (A), 2 µg/L (B), 20 µg/L (C) or 200 µg/L (D). Grey regions represent exposure time. Daphnids were raised to 21 days in clean OECD water post-exposure. Each treatment group was comprised of 30 total individuals exposed in two batches of 15 individuals each. Points represent batch mean ± SEM. Groups differing from the DMSO control are indicated with *, groups differing from the OECD control are indicated with # (Kruskal-Wallis, $p < .05$).

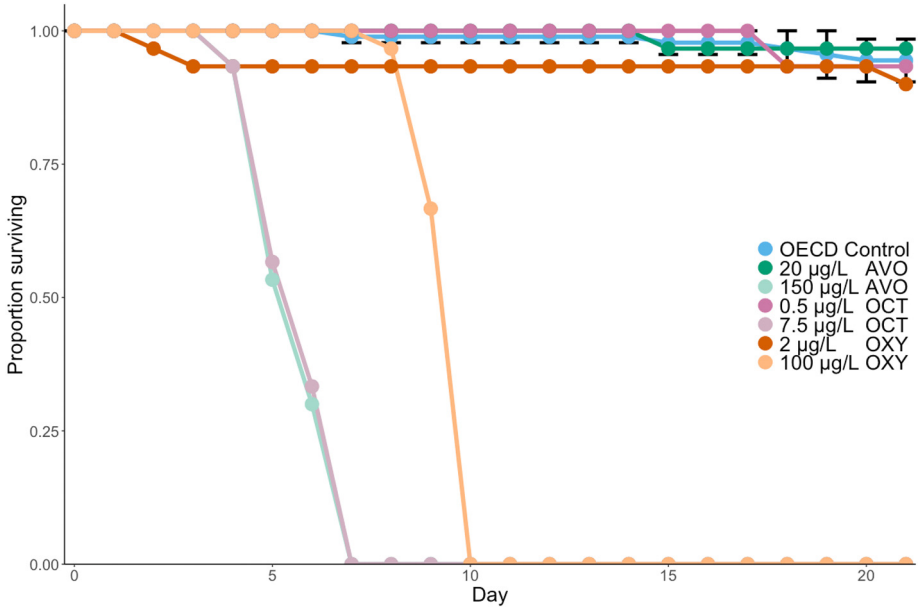

Fig. 5. Survivorship curves of <24-hour old *Daphnia magna* neonates exposed to individual UV filters or an OECD water control for 21 days. Each UV filter treatment group was comprised of 30 total daphnids exposed individually. OECD data are the means of 3 replicates of 30 daphnids. Bars represent mean ± SEM.

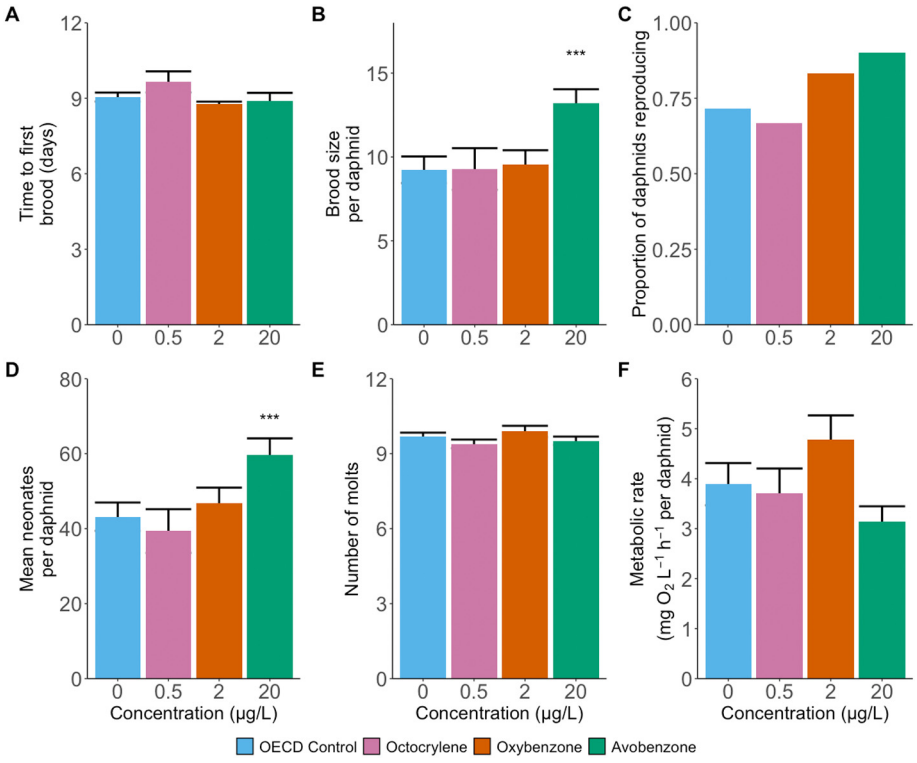

Fig. 6. <24-hour old *Daphnia magna* neonates were exposed to OECD water, or UV filter treatments for 21 days. Individual daphnids were scored daily for the time to first brood (A), average brood size per daphnid (B), the proportion of reproductive adults (C), the number of neonates released per individual (D) and the number of molts (E). Metabolic rate was measured at the end of the 21st exposure day (F). Stars indicate significance with respect to OECD control via Kruskal-Wallis test ($p < .05$).

the "spawn or die" hypothesis (Blewett et al., 2017; Giraudo et al., 2019; Im et al., 2020). Investing a greater proportion of resources into reproductive effort can ensure the continued survival of populations exposed to potentially lethal substances. Producing more neonates per brood and releasing a higher total number of neonates per period of time provides greater opportunity for phenotypic adaptation, particularly in species with short generation times such as this (Chatterjee et al., 2019). Furthermore, the observed AVO-induced increase in reproductive output suggests that AVO is indeed an endocrine disrupting compound, displaying increases in total neonate production similar to daphnids exposed to 10 µg/L estradiol (Xu et al., 2019). Several studies have estimated the estrogenic potencies of benzophenone and other UVFs to be on a similar scale as known xenoestrogens (Witorsch and Thomas, 2010). It is important to note that the reproductive effects of UVF exposure in the present study may not directly translate to potential effects observed in a sexually reproducing species. *D. magna* are a parthenogenic species, therefore differences in toxicity may arise due to the different processes governing the two methods of reproduction (Altshuler et al., 2011).

Alterations to metabolic rate by 25% were observed in chronically exposed daphnids to AVO (negative) and OXY (positive), approaching significance (Fig. 6F). A previous study by Ahn et al. (2019) suggested that AVO is a metabolically-disrupting obesogen, upregulating genes associated with lipid metabolism, cholesterol biosynthesis and metabolic processing of progesterone, in addition to down regulating an equivalent number of genes involving epidermal differentiation and growth regulation. The authors suggest that metabolic disruption should be considered a major outcome of AVO toxicity, a conclusion that the observed decrease in AVO-exposed metabolic rate is in agreeance with. Metabolic disruption has also been noted after exposure to environmental levels of OXY; oxidative stress increased via a reduction of glutathione and an increase in catalase, consistent with the increased generation of free oxygen radicals that would be associated with the increased metabolic rate observed in chronic OXY treatment (Gao et al., 2013).

Understanding the metabolic pathways mediating biotransformation of UVFs is crucial to properly assess the environmental risk posed by contamination, as the mean time to removal as well as the toxicity of metabolic intermediates are important factors influencing overall toxicity. Phase I biotransformation of OXY yields 2,2-dihydroxy-4-methoxybenzophenone, a compound with greater estrogenic ability than OXY (Chen et al., 2017). Little information in this regard is available for OCT and particularly AVO; however, OCT has been shown to undergo carboxylesterase-mediated hydrolysis at a high rate (Saunders et al., 2019). Further research is required to more precisely identify the mechanisms causing the observed physiological responses to UVF exposure, as well as the methods of toxicant removal after uptake.

4.3. Impact on daphnid behaviour

Significant behavioural impairment was observed in daphnids subjected to 200 µg/L AVO or OCT (Fig. 2A). A similar extent of impairment was also observed in the mixture treatments. A previous study on *Daphnia* phototaxis theorized that the two leading causes of a decreased phototactic response are either an impairment of detection through decreased light sensitivity, or a lack of physical capability to respond to said cues (Delompré et al., 2019a). Only 20% of daphnids would be expected to experience a loss of mobility at this concentration of AVO, therefore it is more likely that this substance reduced the ability of *Daphnia* to detect the stimuli in a timely manner. Due to the complete immobilization observed in 200 µg/L OCT treatments during EC_{50} determinations (Fig. 1B), it is probable that a lack of mobility was the cause of the observed behavioural impairment. However, approximately 25% of daphnids in this treatment group proved capable of mobility by completing the phototactic assay within the allotted 5 min, therefore we speculate that partial, immediate recovery of mobility is possible upon

removal of UVF contaminants, as the assay was performed in OECD water. Indeed, rapid behavioural recovery has been noted post-exposure for a variety of contaminants, several of which on the scale of minutes to hours (Jüttner et al., 2010; McWilliam and Baird, 2002). Additionally, complete long-term recovery of behavioural functions has been demonstrated in all tested compounds and concentrations in the present study over a 19-day post-exposure period (Fig. 2B). Continuous exposure is a likely requirement to maintain phototactic inhibition.

Maintaining the ability to properly detect and efficiently respond to light stimuli is of utmost importance to wild daphnids (Brausch et al., 2011). *Daphnia* rely on diel vertical migratory behaviours to navigate water columns throughout the day, a delicate balance of predator avoidance and resource gathering, driven by environmental cues such as light stimuli (Glaholt et al., 2016). Any inability to detect, interpret or respond to these cues increases the risk of an individual being left exposed to predators, and subsequently eliminated from the population (Brausch et al., 2011). Extrapolated to the population level, the observed capability of AVO and OCT to disrupt phototactic behaviour through immobilization and other means poses a widespread threat to populations exposed to UVF contaminants, especially OCT due to the low observed concentration of immobilization. Any impairments that lead to untimely death through either behavioural perturbation or other means are the most toxic of all outcomes.

4.4. Impact on survivorship

None of the tested UVF treatments increased mortality in *Daphnia* neonates during a 48-hour developmental exposure at environmental concentrations (Fig. 4). However, long-term effects are clearly present post-exposure to 200 μg/L AVO, OCT and UVF mixtures, as daphnid survivorship was reduced to 0.63 ± 0.03 in AVO exposures, and 0.13 ± 0.07 in OCT exposures over the following 7 days, with similar results observed with mixture exposures (Fig. 4D). Chronic exposure to high doses of AVO and OXY resulted in complete extermination of daphnid populations in a similar 7-day time period (Fig. 5.) Perhaps most concerning of all, 7 μg/L OCT yielded identical results at concentrations that have been previously observed in surface waters (Schaap and Slijkerman, 2018). The decreased survivorship of UVF-exposed daphnids resulted in a proportional decrease in the cumulative number of neonates produced by each treatment group, including 30% and 80% reductions respectively in acutely exposed AVO and OCT groups (data not shown). Despite surviving *Daphnia* demonstrating recovery following acute developmental exposures, toxicity to populations is evident, and large declines in number are possible in environmental conditions, which can potentially lead to trophic cascades of both predator and prey species of *Daphnia magna*, disrupting the ecosystem as a whole (Li et al., 2019; Miner et al., 2012).

The lack of observable physiological changes in OXY, and particularly OCT treatments in consideration with the overt chronic lethality at 7 μg/L, suggest that the endpoints traditionally observed over a 21-day OECD 211 reproduction test may not be an ideal toxicological assay for this particular class of compounds. Additional emphasis should be placed on biochemical assays focusing on oxidative stress and metabolic regulatory pathways, as these have been shown to be directly disrupted by UV-filter exposure (Ahn et al., 2019; Cocci et al., 2020; Rodríguez-Fuentes et al., 2015). Ecologically relevant behaviours should also be a prime candidate for experimentation, as our study demonstrates that acute AVO and OCT exposure results in decreased phototactic response.

4.5. Environmental implications

These results are promising in the context of environmental remediation. OCT and OXY half-lives are estimated to be on the scale of several days in surface waters up to several months, dependant on environmental parameters (Liu et al., 2013; Rodil et al., 2009; Semones et al., 2017; Vione et al., 2013). A reduction of contaminant inputs into the aquatic environment can ensure that UVF concentrations decrease below levels of concern without the need for active removal from the waters. This can be achieved through improved WWTP techniques, as current technologies suffer incomplete removal efficiency of many organic UVFs, and as such are a primary source of these compounds in the environment (Butkovskyi et al., 2016; Ma et al., 2019; O'Malley et al., 2019). The present results suggest that behavioural disruption of *Daphnia* migratory behaviours is temporary and can be recovered over several weeks. Compounds such as AVO that alter reproductive patterns under chronic conditions do not exhibit similar effects in acutely exposed neonates. While it is possible that complete recovery can occur within the same generation post-exposure, further testing is required to ensure that lingering effects do not occur in post-exposure generations, as UVFs are known bioaccumulators (Cocci et al., 2020; Gago-Ferrero et al., 2013a; He et al., 2019).

The vast majority of UVF-containing plastics and commercially available sunscreens utilize a combination of UVF active ingredients in order to offer a more broad protection spectrum (Hanson et al., 2020; Muncke, 2011), therefore mixtures of several unique UV filters offer a more environmentally representative perspective of aquatic exposures. The data in the present study suggest that a 1:1:1 mixture of AVO, OCT and OXY does not alter the level of toxicity to daphnid neonates to any meaningful degree with respect to its individual components, as the mixture effects for each observed endpoint were highly comparable to the most toxic UVF (Ex: Fig. 1D-G, Fig. 2A, Fig. 4D). This is consistent with previous studies of various UVF mixtures in aquatic organisms and invertebrates, indicating that direct interactive effects between multiple UVFs are generally minimal, and mixture toxicity is dictated by the concentration of the most toxic component (González and Gui, 2018; Mao et al., 2018). It should be noted that UV stabilizers are another core component of commercial products due to their ability to promote UVF stability, therefore future research should consider these components in studies of complex mixture effects (Peng et al., 2020).

5. Conclusions

By far the most well-characterized compound present in this study, OXY proved to be the least toxic of the studied UV-filters, increasing metabolic rate in chronic 2 μg/L exposures. AVO also induced metabolic disruption, as well as significantly increased reproductive output of *Daphnia magna*. Phototactic ability was severely reduced in 48-hour 200 μg/L acute exposures to AVO and OCT, due to either reduced ability to detect light stimuli or lacking physical capability to efficiently respond. Perhaps the least studied of the tested compounds, OCT appeared to be the most toxic, causing a high degree of immobilization near environmental concentrations, in addition to proving highly lethal chronically at 7.5 μg/L. An overall absence of detectable responses in 21-day physiological assays following acute developmental exposures to high doses of UV-filters suggests that additional experimentation is required, as delayed mortality was observed post-exposure in all UVF treatments except OXY. Overall, the results suggest that UV-filters are capable of biologically and ecologically relevant disruptions to *Daphnia magna* at environmentally realistic concentrations.

CRediT authorship contribution statement

Aaron Boyd: Conceptualization, Investigation, Formal analysis, Data curation, Writing - original draft. **Connor B. Stewart:** Investigation, Data curation, Writing - review & editing. **Danielle A. Philibert:** Conceptualization, Writing - review & editing. **Zuo Tong How:** Investigation, Writing - review & editing. **Mohamed Gamal El-Din:** Resources, Writing - review & editing. **Keith B. Tierney:** Resources, Writing - review & editing, Supervision. **Tamzin A. Blewett:** Conceptualization, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known financial or personal relationship conflicts of interest.

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