The Effects of Salinity and Acetaminophen on the Aquatic Snail *Physa acuta*

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Pharmaceuticals are increasingly detected in water bodies, and their presence can negatively impact aquatic organisms. This effect can be amplified when combined with increasing salinity in freshwater ecosystems. Acetaminophen is a widely used analgesic that is commonly found in river, streams, and waters where it is discharged directly. Therefore, organisms present in these locations (e.g., insects, snails, amphibians, and fish) are likely to be affected by acetaminophen. In this study, we determined the effects of elevated salinity (0.68 g/L), acetaminophen (500 µg/L) and combined elevated salinity (0.68 g/L) and acetaminophen (500 µg/L), on the growth, reproduction, and movement of the freshwater snail *Physa acuta*. There were no effects on growth or reproduction. No changes were observed on movement in individual treatments groups; however, there was a significant effect in the combined treatment of salinity and acetaminophen. It is likely that an energetic trade-off between physiological mechanisms resulted in a synergistic negative effect on snails.

Introduction

Pharmaceuticals have been produced since the 19th century (Drews, 2000) and were initially manufactured in small quantities (Taylor, 2015). In recent years, pharmaceuticals production has increased dramatically in response to human population growth and corresponding increasing of food consumption, and a rise in demand for humans and veterinary medicines (Kola and Landis, 2004). Humans use pharmaceuticals in everyday life, and in livestock and pets targets animal diseases. After consumption or application, these compounds are excreted in urine and feces (Calisto & Esteves, 2009) where they eventually reach wastewater treatment plants (humans) and open ponds or lagoons (animals).

Wastewater is treated in treatment plants through different processes including addition of chlorine and chlorine dioxide, adsorption/ bioadsorption on activated carbon, advanced oxidation processes, photooxidation, and electrochemical processes (Rivera-Utrilla et al., 2013). However, wastewater treatment plants have proven ineffective in completely removing pharmaceuticals (Gros et al., 2010; Zur et al., 2018). Animal waste is stored in open ponds or lagoons where they are later spread to farm fields as fertilizers. This waste can reach aquatic ecosystems and drinking water sources becoming a non-point source of pollution via runoff and leaching (Nikolaou et al., 2007) if not properly managed or treated. Thus, because of increased production and usage of pharmaceuticals, ineffective wastewater treatments, and polluted water from animal agriculture, these chemicals are commonly detected in aquatic ecosystems (Nikolaou et al., 2007; Taylor, 2015; Ngqwala & Muchesa, 2020; Ortuzar et al., 2022).

There are many pharmaceuticals detected in water (Table 1; Wilkinson et al., 2022). For example, a multiagency effort conducted in 2016 to 2017 throughout the U.S, detected in urban stormwater samples, five nonprescription pharmaceuticals, caffeine, nicotine, acetaminophen, cotinine, and lidocaine, as well as two prescription pharmaceuticals, metformin and methanamine (Masoner et al., 2019). Further, from 2014 to 2017 in four regions across the U.S., over 100 pharmaceuticals were detected in 91% of 308 sampled streams (Bradley et al., 2020). Previously, as part of the Environmental Protection Agency's (USEPA's) National Rivers and Streams Assessment, samples were collected in 2007 and 2008. In these samples, sulfamethoxazole was the most frequently detected in 77.5% of 182 surface water samples (Batt et al., 2015). In Taiwan, 86% of 97 target compounds were detected in 23 water sites. Sulfamethoxazole, caffeine, acetaminophen, and ibuprofen were detected in over 91% of samples with median maximum concentrations exceeding 0.02 µg/L (Lin et al., 2008). In China, 45 pharmaceuticals were found in 217 water samples (~21%). In these samples, trimethoprim, caffeine, flumequine, metformin, triclocarban, and carbamazepine had a detection frequency greater than 90% and a mean concentration of over 4.9 ng/L (Yao et al., 2018). Most of the detected pharmaceuticals are classified as analgesics, anti-inflammatories, antibiotics, beta blockers, endocrine disruptors, hormones and steroids, lipid regulators, stimulants, or psychiatric drugs (Monteiro & Boxall, 2010).

Table 1. Commonly detected pharmaceuticals in natural waters (Adeleye et al., 2022).

Compound	Concentration (ng/L)	
	min	max
Atenolol	0.25	1237
Metoprolol	0.5	3960
Acetaminophen	1	107,000
Aspirin	0.5	22,900
Codeine	1.5	529
Diclofenac	0.42	57,160
Ibuprofen	1.2	84,600
Indomethacin	1.21	111
Ketoprofen	0.74	620
Mefanamic	0.02	541
Naproxen	0.3	12,300
Salicylic Acid	0.3	5,170
Tramadol	30	5,970
Chloramphenicol	1.06	660
Ciprofloxacin	0.56	13,567
Erythromycin	0.02	362
Florfenicol	1.6	2,840
Tetracycline	5	712
Trimethoprim	0.13	13,600
Sulfamethoxazole	0.21	38,850
Norfloxacin	0.2	572
Gemfibrozil	0.25	1,115
Clofibric	0.01	450
Caffeine	11	144,179
Carbamazepine	1	11,581

Acetaminophen (N-Acetyl-4-aminophenol) is one of the most commonly used pharmaceuticals due to its analgesics (pain relieving) and antipyretics (fever reducing) properties. Over 200 approved products (e.g., Tylenol, Paracetamol, Panadol) contain acetaminophen as the main ingredient (Blough & Wu, 2011). Acetaminophen's mechanism of action in humans is the inhibition of prostaglandin synthesis by blocking cyclooxygenase (COX) enzymes, which results in the blocking of pain (Gunaydin & Bilge, 2018). Worldwide (Wilkinson et al., 2022), acetaminophen has been detected at maximum concentrations of 73 ng/L (South Korea; Kim et al., 2007), 298 ng/L (Canada; Kleywegt et al., 2011), 339 ng/L (China; Yang et al., 2013), and 7 ng/L (Germany and Sweden; Li et al., 2016). In the U.S., acetaminophen concentrations in water ranges from ng/L to µg/L; with peak concentrations in streams of ~10 µg/L (Kolpin et al., 2002) to 220 µg/L (Yu et al., 2013), and 3274 µg/L in wastewater (Pharms UBA, 2021). Peak concentrations of acetaminophen vary depending on environmental conditions and removal efficiency of wastewater treatment plant. Once acetaminophen enters aquatic ecosystems and drinking water sources, it has the potential to induce negative effects on biota and human health.

The effects of acetaminophen on aquatic organisms might result from oxidative stress through inhibition of acetylcholinesterase oxidation and neurotoxicity (Almeida & Nunes, 2019). For example, acetaminophen may interfere with embryonic development, reproduction, growth, behavior, and endocrine function (David & Pancharatna, 2009, Trappe et al., 2011, and Keaveney et al., 2020). Specifically, acetaminophen (30 µg/L) has caused a decrease in fish liver glycogen and swimming speed (Choi et al., 2018). Acetaminophen might also affect spawning in invertebrates (e.g., mussels) by inhibiting prostaglandins synthesis (Sole et al., 2010). For marine snails, such as *Phorcus lineatus*, long term exposure to acetaminophen induced changes in catalase, acetylcholinesterase, and pseudocholinesterase activity. These changes might result in adverse effects on respiration, feeding, and behavior (Almeida and Nunes, 2019).

Physa acuta (bladder snail) is a species of air-breathing freshwater gastropod mollusk. They are hermaphroditic with both male and female reproductive organs and are capable of self-fertilization. *P. acuta* is present throughout most of the United States (Ebbs et al., 2018). They usually inhabit freshwater rivers, lakes, streams, swamps, and ponds with relatively warm waters. *P. acuta* is an omnivore with a diet of decaying plants, algae, and insects. These snails have a critical role in energy flow and nutrient cycling acting as links in the food web between sediment microbial communities and higher trophic levels (Justice & Bernot, 2014; Elias & Bernot, 2017).

Anthropogenic activities, such as agriculture, usage of road salt, and saltwater intrusion have altered the concentration and composition of ions in bodies of water (Schafer et al., 2011; Tiwari et al., 2018; Corwin, 2020). Increasing salinity disrupts organisms' metabolism and water balance (Canedo-Arguelles et al., 2018). Water salinity influences the toxicity of pollutants in marine ecosystems (DeLorenzo et al., 2009), and it may affect species' sensitivity to pollution (Velasco et al., 2019). Common stressors paired with salinity are temperature and metals (Alutoin et al., 2001; Heugens et al., 2008; Velasco et al., 2019), but little is known about the combined effects of elevated salinity and pharmaceuticals in freshwater ecosystems. As sea levels rise and the human population expands, saltwater intrusion and pharmaceutical pollution become more urgent issues.

This ecotoxicological study assessed the interaction between elevated salinity and acetaminophen exposure on *Physa acuta*. Freshwater gastropods have quantifiable behavioral changes in response to chemicals in the water, which makes them an ideal test organism for studies that aim to understand the effects of emerging contaminants in aquatic and terrestrial ecosystems (Elias & Bernot, 2017; Schuijt et al., 2021). This research assesses the chronic effects of acetaminophen exposure and elevated salinity to *P. acuta*. We predict that higher salinity and combined levels of salinity and acetaminophen will reduce the growth of exposed snails. Due to neurotoxicity, we predict decreased movement of snails exposed to acetaminophen and decreased movement of snails at higher salinity due to osmotic stress. Additionally, number of eggs masses, and number of eggs are expected to decreased due to reduced overall fitness.

Methods

Physa acuta were purchased from Carolina Biological Supply. The snails were kept in a ~40 L aquarium filled with aerated and de-chlorinated tap water for 10 days. Snails were fed boiled spinach *ad libitum*. and maintained under a 16:8 light:dark photoperiod for the duration of the experiment. Test tubes (25 mL) were used to keep individual snails in 20 mL of test solution. Four treatments with eight replicates each (n = 32) addressed the individual and combined effects of environmentally relevant concentrations of acetaminophen and salinity. Normal salinity refers to freshwater salinity (0.18 g/L). Treatments were: control (0 µg/L acetaminophen; normal salinity), acetaminophen (500 µg/L), elevated salinity (0.68 g/L), and acetaminophen x elevated salinity (500 µg/L x 0.68 g/L).

Stock solutions were prepared for acetaminophen and salinity to achieve concentrations of 10 mg/L for acetaminophen and 0.68 g/L for salinity. Aliquots from each stock solution were added to test tubes to reach target concentrations. Acetaminophen concentration was not confirmed through analytical methods. Salinity was measured using a pH/ Conductivity/TDS/Salinity PC60 tester (Apera Instruments). Acetaminophen concentration was selected to represent relevant concentrations of acetaminophen detected in the U.S. and globally (Pharms UBA, 2021). Salinity concentration was selected to mimic saltwater intrusion into freshwater ecosystems (Abd-Elhamid & Javadi, 2008). Water changes for treatment renewal and feeding (spinach *ad libitum*.) were conducted every other day (~3 times a week). Snail shell length, movement, and total number of eggs in each egg mass were measured weekly.

Experimental Design. Experiment set up consisted of *Physa acuta* and four different treatments (control, salinity, acetaminophen, and acetaminophen x salinity) with eight replicates per treatment (n = 32). Snail shell length was recorded at the beginning and end (14 days) of the experiment. Shell length was measured using ImageJ. Snail movement was quantified by replicating the methods of Bernot et al., 2005, and Elias and Bernot, 2017. Snail movement change was calculated as the difference between day 7 and day 14. For each movement measurement, individual snails were placed in a glass aquarium (50.8 x27.9 x 30.5 cm) filled with aerated and dechlorinated tap water to a height of 5 cm. Under the aquarium, a 1 cm^2 square grid paper was placed. Snails were set aperture down on the bottom of the aquarium in the center of the grid paper. After allowing 10 seconds for acclimation, snail movement was recorded as the number of grid lines crossed within 1 minute. Other testing protocols used 10 days (e.g., Ma et al., 2010), 14 days (e.g., Kefford and Nugegoda, 2004), 21 days (e.g., Aquilino et al., 2018), and 28 days (e.g., Bernot and Brandenburg, 2013) to address chronic or long -term effects of contaminants on freshwater snails. Thus, our experiment duration of 14 days provides a starting assessment on changes in growth and movement that might support lowest observed effect concentration endpoints.

Data Analysis. Differences in growth, movement, and number of eggs, across treatments were evaluated using Analysis of Variance when data were normally distributed or Kruskal-Wallis test when data were not normally distributed. Tukey post-hoc or Dunn test was used to identify pair-wise differences when an overall difference was found. Normality was assessed using Shapiro-Wilks test and homogeneity of variance was assessed using Bartlett's test. All analyses were conducted in R 4.2.1 (R Core Team 2022). Alpha was set to 0.05 for all analyses.

Results

***Physa acuta* growth.** Growth after two weeks was normally distributed within each group (all $p > 0.05$) and variances were equal across groups ($K^2 = 3.454$, df = 3, $p = 0.330$). No statistically significant differences in growth across treatment groups were found ($F_{[3,15]} = 1.011$, $p = 0.415$). Snail growth in length ranged from 0.6 mm (acetaminophen x salinity) to 1.08 mm (salinity) after 14 days (Fig. 1A, Table 2). Snails exposed to higher salinity had a smaller increase of shell length of 24% compared to the control. In contrast, snails exposed to combined salinity and acetaminophen had a larger change of shell length of 31% compared to the control.

***Physa acuta* egg mass/egg production.** Total number of eggs across treatments were normally distributed (all $p > 0.05$) and variances were equal ($K^2 = 0.274$, df = 3, $p = 0.965$). No significant differences in the total number of eggs were found ($F_{[3,15]} = 0.124$, $p = 0.945$). On average, total number of eggs ranged from 6.5 to 13.75 (Fig. 1B, Table 2). Specifically, snails produced ~2 x more eggs than control (6.5 eggs) when exposed to acetaminophen (13.75 eggs), 1.05 x more eggs than control when exposed to salinity (6.88 eggs), and 1.56 x more eggs than control when exposed to combined conditions (10.13 eggs). Egg mass production was greater across treatments.

***Physa acuta* movement rate.** Change in movement were normally distributed (all $p > 0.05$) and variances were equal across groups ($K^2 = 3.503$, df = 3, $p = 0.320$). An overall significant difference in movement across treatment groups was identified ($F_{[3,15]} = 3.484$, $p = 0.043$; Fig. 1B, Table 2). Overtime, snail movement decreased for control (-6.0 cm/ min), individual acetaminophen (-0.79 cm/min), and individual salinity (-4.0 cm/min) treatments. Specifically, the combination of acetaminophen and salinity was significantly different from the control ($p = 0.043$) and movement increased by 116% (i.e., the snails moved more than control snails).

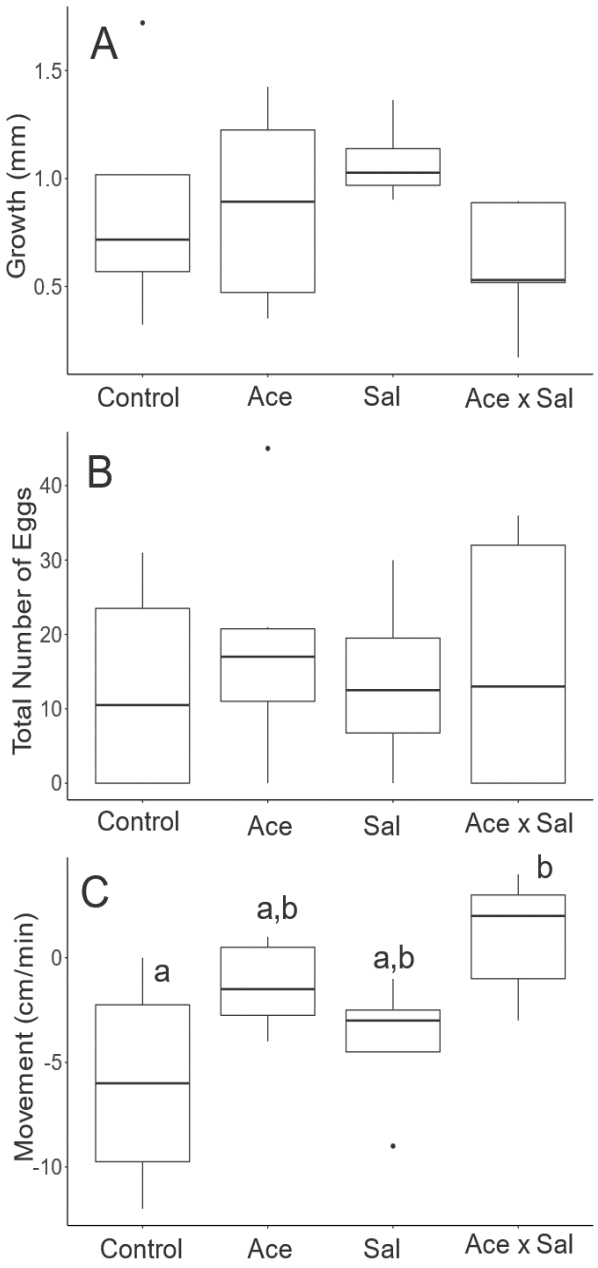

Figure 1: Changes on *Physa acuta* growth, total number of eggs, and movement after 14 days exposure to acetaminophen, salinity, or acetaminophen and salinity. *Physa acuta* growth (A), total number of eggs (B), and movement (C) after 14 days exposure to individual acetaminophen (Ace) and salinity (Sal), and combined acetaminophen and salinity treatments. There were not effects (Sal, Ace, and Ace x Sal) on snail growth and number of eggs, or individual effects on movement. Combined acetaminophen and salinity (Ace x Sal) had a significant effect on snail movement.

Table 2. Changes on *Physa acuta* growth, total number of eggs, and movement after 14 days exposure to acetaminophen, salinity, or acetaminophen and salinity. *Physa acuta* growth, total number of eggs, and movement after exposure to (0 µg/L acetaminophen; normal salinity), acetaminophen (ACE; 500 µg/L), salinity (Sal; 0.68 g/L), and acetaminophen x salinity (ACE x Sal; 500 µg/L x 0.68 g/L). Movement was measured as described in Elias and Bernot, 2017 and reported as the difference across 7 and 14 days of exposure.

Treatment	Growth (mm)	Total # of egg masses	Movement (cm/min)
Control	0.87	6.5	-6.00
ACE	0.87	13.75	-0.79
Sal	1.08	6.88	-4.00
ACE x Sal	0.60	10.13	1.00

Discussion

In this research, we assessed the effects of individual and combined effects of salinity and acetaminophen on *Physa acuta*. The endpoints chosen, growth, egg production, and movement are important indicators of snail fitness. The selected treatments of salinity and acetaminophen represent increased salinity of natural freshwater systems and the continuously growing prevalence of pharmaceuticals entering waterways (Love et al., 2008; Adeleye et al., 2022). Increasing salinity of freshwater habitats is a likely scenario to happen due to global climate change (Dasgupta et al., 2015). Further, other research has addressed the effects of multiple stressors on aquatic snails including multiple pesticides (Elias & Bernot, 2017), metals and contraceptive drugs (Völker et al.,2014), metals and fungicides (Gnatyshyna et al., 2020), antiepileptic drugs and herbicides (Heye, 2019), and insecticide and salinity (Heugens et al., 2008). However, little is to be found on the combined effects of salinity and acetaminophen on freshwater ecosystems.

Snail growth and reproduction did not show significant changes. None of the treatments, individual or combined, had a significant effect on *Physa acuta* shell length or egg production. Specifically, when exposed to higher salinity (0.68 g/L), snail growth did not significantly change. This response was similar to Kefford and Nugegoda, 2005, where no changes in growth and egg production were observed from 50 µS/cm (~0.0275 g/L) to 5000 µS/cm (~2.75 g/L). These authors concluded that increasing salinity might actually increase growth and egg production until a maximum salinity is reached. Detrimental effects are likely expected pass this maximum (Kefford &Nugegoda, 2005). Additionally, *P. acuta* is likely to have high salinity tolerance. Concentrations ranging from 0.2 g/L to 4.2 g/L had no effect on survival of juveniles and adult snails (Zukowski & Walker, 2009). Similar salinity tolerance was observed for *Physa pomilia* (0.14 g/L to 2 g/L), with no effects on egg production (Suski et al., 2012). Changes on snail reproductive effort, measured as egg production, decreased at salinity levels of 7 g/L (Stockwell et al., 2011). While *P. acuta* is tolerant to laboratory concentrations exceeding 4 g/L; this species avoids water bodies with salinity above 2.6 g/L in natural habitats (Cieplok & Spyra, 2020).

Overall, acetaminophen did not have an effect on snail growth and egg production. It is likely that analyzes of specific biomarkers could provide better physiological evidence of acetaminophen sublethal effects (Bhagat et al., 2016). For example, oxidative effects of acetaminophen were observed on aquatic snails by other researchers (e.g., Alma, 2016; Almeida & Nunes, 2019; Gimenez & Nunez, 2019). This oxidative stress can be measured by quantifying malondialdehyde in tissue or catalase enzymatic activity (Bhagat et al., 2016). At elevated concentrations of acetaminophen such as those find near hospital effluent (51,600 µg/L), tissue damage in *P. acuta* was observed after 30 days exposure (Sobrino-Figueroa, 2015). For saltwater snails such as *Phorcus lineatus* and *Gibbula umbilicalis*, catalase activity significantly increased after acetaminophen exposure of 5 µg/L for 28 days (Almeida & Nunes, 2019) and 5 µg/L for 96 hours (Giménez & Nunes, 2019).

Fitness related traits (e.g., egg production) as well as vigor related traits (e.g., movement) are good indicators of organism health (Coutellec & Lagadic, 2006). In the presence of environmental stressors such as physicochemical parameters (e.g., pH and salinity) and pollutants (e.g., pesticides, metals, and pharmaceuticals), there is a decrease in snail fitness (Bernot et al., 2005; Justice & Bernot, 2014; Elias & Bernot, 2017). In our study, we quantified snail movement to determine organismal fitness when exposed to individual and combined salinity and acetaminophen. Similar to growth and egg production, there was not effect of individual salinity and acetaminophen on movement. However, we observed a significant effect on movement when snails were exposed to the combined treatment.

Increasing salinity affects permeability and osmotic balance in freshwater biota. These changes of metabolic stability can lead to cellular damage and corresponding effects on fitness (Cañedo-Argüelles et al., 2018). Additionally, salinity can alter the physical and chemical properties of pharmaceuticals (Almeida et al., 2022). While the salinity level (0.68 g/L) used in this research might have not elicited a change in growth, egg production, and movement, it might have induced physiological mechanisms that control osmotic and dehydration stress which requires energy (Bradley, 2009; Rivera & Lignot, 2017). Further, it is also likely that cellular mechanisms such as antioxidant activity were present in response to oxidative stress of acetaminophen exposure. Thus, if there were energetic exchanges between these physiological protective mechanisms, exposure to one stressor (i.e., elevated salinity or acetaminophen) compromised the response to the other stressor resulting in a synergistic negative effect (Velasco et al., 2019) and the observed significant change on snail movement.

Exposure to combined salinity and acetaminophen had direct effects on snail movement as well as potential indirect effects to food webs and ecological interactions. Changes in *P. acuta* movement could affect antipredator behavior and avoidance from predatory organisms (Justice and Bernot, 2014). *Physa acuta* feeds on biofilm growing on organic and inorganic materials (Brady & Turner, 2010). Thus, changes on movement could affect feeding behavior and their role as primary consumers and decomposers (Ruetz et al.,2002). In addition to quantifying organisms' fitness through growth, egg production, and movement, future research should include biomarkers to match or better explain physiological changes associated with such endpoints.

Conclusions

Overall, our hypotheses were partially supported. We did not observed changes in snail growth, reproduction, and movement when exposed to individual acetaminophen and elevated salinity. However, we observed significant changes in movement when exposed to combined elevated salinity and acetaminophen. This study shows that exposure to multiple stressors have the potential to cause synergistic effects. It is likely that the energy cost of the protective mechanisms from oxidative stress (acetaminophen) and osmotic balance (elevated salinity), compromised the response of one stressor over the other. Quantifying biomarkers of these mechanisms would provide better understanding of the behavioral changes (movement) observed in this research. Further, changes in snail movement can be linked to changes in food web and nutrient cycling. Thus, there is a need to continue exploring the effects of multiple stressors on aquatic organisms.

Notes and References

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Abd-Elhamid HF, Javadi AA. 2008. An investigation into control of saltwater intrusion considering the effects of climate change and sea level rise. Proceeding of 20th Salt Water Intrusion Meeting, Florida, USA.

Adeleye AS, Xue J, ZhaoY, Taylor AA, Zenobio JE, Sun Y, Han Z, Salawu OA, Zhu Y. 2022. Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. *Journal of Hazardous Materials*, *424*, p.127284.

Almeida F, Nunes B. 2019. Effects of acetaminophen in oxidative stress and neurotoxicity biomarkers of the gastropod Phorcus lineatus. Environmental Science and Pollution Research. 26: 9823-9831. https://doi.org/10.1007/s11356-019-04349-1

Almeida Â, Calisto V, Esteves, VI, Schneider RJ, Soares AM, Freitas R. 2022. Salinity-dependent impacts on the effects of antiepileptic and antihistaminic drugs in Ruditapes philippinarum. Science of the Total Environment, 806, 150369.

Aquilino M, Martínez-Guitarte JL, García P, Beltrán EM, Fernández C, Sánchez-Argüello P. 2018. Combining the assessment of apical endpoints and gene expression in the freshwater snail Physa acuta after exposure to reclaimed water. Science of the total environment, 642, 180-189.

Batt A, Kincaid TM, Kostich MS, Lazorchak JM, Olsen AR. 2016. Evaluating the extent of pharmaceuticals in surface waters of the United States using a National-scale Rivers and Streams Assessment survey. Environmental Toxicology and Chemistry, 35(4), 874-881.

Bernot RJ, Brandenburg M. 2013. Freshwater snail vital rates affected by non-lethal concentrations of silver nanoparticles. Hydrobiologia, 714(1), 25-34.

Bernot RJ, Kennedy EE, Lamberti GA. 2005. Effects of ionic liquids on the survival, movement, and feeding behavior of the freshwater snail, Physa acuta. Environmental Toxicology and Chemistry: An International Journal, 247, 1759-1765.

Bhagat J, Ingole BS, Singh N. 2016. Glutathione S-transferase, catalase, superoxide dismutase, glutathione peroxidase, and lipid peroxidation as biomarkers of oxidative stress in snails: A review. Invertebrate Survival Journal, 131, 336-349.

Blough ER, Wu M. 2011. Acetaminophen: beyond pain and Fever-relieving. Frontiers in pharmacology. 2, 72. https://doi.org/10.3389/fphar.2011.00072

Bradley PM, Journey CA, Button DT, Carlisle DM, Huffman BJ, Qi SL, Romanok KM, Van Metre PC. 2020. Multi-region assessment of pharmaceutical exposures and predicted effects in USA wadeable urban-gradient streams. PLoS One, 151, e0228214.

Bradley TJ. 2009. Animal osmoregulation. Oxford University Press.

Brady JK, Turner AM. 2010. Species-specific effects of gastropods on leaf litter processing in pond mesocosms. Hydrobiologia, 6511, 93-100.

Calisto V, Esteves VI. 2009. Psychiatric pharmaceuticals in the environment. Chemosphere, 7710, 1257-1274.

Cañedo-Argüelles M, Kefford B, Schäfer R. 2019. Salt in freshwaters: causes, effects and prospects-introduction to the theme issue. Philosophical Transactions of the Royal Society B, 374(1764), p.20180002.

Choi E, Alsop D, Wilson JY. 2018. The effects of chronic acetaminophen exposure on the kidney, gill and liver in rainbow trout Oncorhynchus mykiss. Aquatic Toxicology, 198, 20-29.

Cieplok A, Spyra A. 2020. The roles of spatial and environmental variables in the appearance of a globally invasive Physa acuta in water bodies created due to human activity. Science of the Total Environment, 744, 140928.

Corwin DL, Yemoto K. 2020. Salinity: Electrical conductivity and total dissolved solids. Soil science society of America journal, 845, 1442-1461.

Coutellec MA, Lagadic L. 2006. Effects of self-fertilization, environmental stress and exposure to xenobiotics on fitness-related traits of the freshwater snail Lymnaea stagnalis. Ecotoxicology, 152, 199-213.

Dasgupta S, Akhter Kamal F, Huque Khan Z, Choudhury S, Nishat A. 2015. River salinity and climate change: evidence from coastal Bangladesh. In World Scientific Reference on Asia and the World Economy pp. 205-242.

David A, Pancharatna K. 2009. Effects of acetaminophen paracetamol in the embryonic development of zebrafish, Danio rerio. Journal of applied toxicology, 29(7), pp.597-602. doi: 10.1002/jat.1446. PMID: 19533668.

DeLorenzo ME, Wallace SC, Danese LE, Baird TD. 2009. Temperature and salinity effects on the toxicity of common pesticides to the grass shrimp, Palaemonetes pugio. Journal of environmental science and health, Part B, 445, 455-460.

Drews, J., 2000. Drug discovery: a historical perspective. science, 287(5460), pp.1960-1964.

Ebbs ET, Loker ES, Brant SV. 2018. Phylogeography and genetics of the globally invasive snail Physa acuta Draparnaud 1805, and its potential to serve as an intermediate host to larval digenetic trematodes. BMC Evolutionary Biology. 18103:1-17.

Elias D, Bernot MJ. 2017. Effects of Individual and Combined Pesticide Commercial Formulations Exposure to Egestion and Movement of Common Freshwater Snails, Physa acuta and Helisoma anceps. The American Midland Naturalist. 1781: 97-111. https://doi.org/10.1674/0003-0031-178.1.97

Giménez V, Nunes B. 2019. Effects of commonly used therapeutic drugs, paracetamol, and acetylsalicylic acid, on key physiological traits of the sea snail Gibbula umbilicalis. Environmental Science and Pollution Research, 2621, 21858-21870.

Gnatyshyna L, Falfushynska H, Stoliar O, Dallinger R. 2020. Preliminary study of multiple stress response reactions in the pond snail Lymnaea stagnalis exposed to trace metals and a thiocarbamate fungicide at environmentally relevant concentrations. Archives of environmental contamination and toxicology, 791, 89-100.

Gros M, Petrović M, Ginebreda A, Barceló D. 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environment international, 361, 15-26.

Gunaydin C, Bilge SS. 2018. Effects of Nonsteroidal Anti-Inflammatory Drugs at the Molecular Level. Eurasian J Med. 502: 116-121. doi: 10.5152/eurasianjmed.2018.0010

Heugens EH, Hendriks AJ, Dekker T, Straalen NMV, Admiraal W. 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Critical reviews in toxicology, 313, 247-284.

Heye K, Graumnitz S, Rybicki M, Schür C, Völker J, Wick A, Oehlmann J, Jungmann D, Oetken M. 2019. Laboratory-to-field extrapolation: Increase in carbamazepine toxicity in a higher tier, multiple-stress experiment. Ecotoxicology and Environmental Safety, 183, p.109481.

Justice JR Bernot RJ. 2014. Nanosilver inhibits Freshwater Gastropod Physa acuta Ability to Assess Predation Risk. The American Midland Naturalist, 171(2), pp.340-349.

Keaveney A, Peters E, Way B. 2020. Effects of acetaminophen on risk taking. Social Cognitive and Affective Neuroscience. 157:725–732, https://

doi.org/10.1093/scan/nsaa108
Kefford BJ, Nugegoda D. 2005. No evidence for a critical salinity threshold for growth and reproduction in the freshwater snail Physa acuta. Environmental pollution, 1343, 377-383.
Kim SD, Cho J, Kim IS, Vanderford BJ, Snyder SA. 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. Water research, 415, 1013-1021.
Kleywegt S, Payne, M, Ng F, Fletcher T. 2019. Environmental loadings of active pharmaceutical ingredients from manufacturing facilities in Canada. Science of the Total Environment, 646, 257-264.
Kola I, Landis J. 2004. Can the pharmaceutical industry reduce attrition rates? Nature Reviews Drug Discovery. 38: 711–716. https://doi.org/10.1038/nrd1470
Kolpin DW, Furlong, ET, Meyer, MT, Thurman, EM, Zaugg, SD, Barber, LB, Buxton, HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: A national reconnaissance. Environmental science technology, 366, 1202-1211.
Li Z, Sobek A, Radke M. 2016. Fate of pharmaceuticals and their transformation products in four small European rivers receiving treated wastewater. Environmental science technology, 5011, 5614-5621.
Lin AYC, Yu TH, Lin CF. 2008. Pharmaceutical contamination in residential, industrial, and agricultural waste streams: risk to aqueous environments in Taiwan. Chemosphere, 741, 131-141.
Love JW, Gill J, Newhard JJ. 2008. Saltwater intrusion impacts fish diversity and distribution in the Blackwater River drainage Chesapeake Bay watershed. Wetlands, 284, 967-974.
Ma T, Gong S, Zhou K, Zhu C, Deng K, Luo Q, Wang Z. 2010. Laboratory culture of the freshwater benthic gastropod *Bellamya aeruginosa* (Reeve) and its utility as a test species for sediment toxicity. Journal of Environmental Sciences, 22(2), 304-313.
Masoner JR, Kolpin DW, Cozzarelli IM, Barber LB, Burden DS, Foreman WT, Bradley PM. 2019. Urban stormwater: An overlooked pathway of extensive mixed contaminants to surface and groundwaters in the United States. Environmental science & technology, 53(17), 10070-10081.
Monteiro SC, Boxall A. 2010. Occurrence and fate of human pharmaceuticals in the environment. Reviews of environmental contamination and toxicology, 53-154.
Ngqwala NP, Muchesa P. 2020. Occurrence of pharmaceuticals in aquatic environments: A review and potential impacts in South Africa. South African Journal of Science, 1167:1-7. doi:http://dx.doi.org/10.17159/sajs.2020/5730
Nikolaou A, Meric S, Fatta D. 2007. Occurrence patterns of pharmaceuticals in water and wastewater environments. Analytical and bioanalytical chemistry, 3874, 1225-1234.
Ortúzar M, González López JJ, Aranda Ballesteros E. 2022. Pharmaceutical Pollution in Aquatic Environments: A Concise Review of Environmental Impacts and Bioremediation Systems.
Pharms UBA Umwelt Bundesamt, "Database- Pharmaceuticals in the environment" Umwelt Bundesamt. 2021. https://www.umweltbundesamt.de/en/database-pharmaceuticals-in-the-environment-0. Accessed 20 September 2022
R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
Rivera-Ingraham GA, Lignot JH. 2017. Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: raising the questions for future research. Journal of Experimental Biology, 220(10), pp.1749-1760.
Rivera-Utrilla J, Sánchez-Polo M, Ferro-García MÁ, Prados-Joya G, Ocampo-Pérez R. 2013. Pharmaceuticals as emerging contaminants and their removal from water. A Review. Chemosphere. 937: 1268–1287. https://doi.org/10.1016/j.chemosphere.2013.07.059
Ruetz CR, Newman RM, Vondracek B. 2002. Top-down control in a detritus-based food web: fish, shredders, and leaf breakdown. Oecologia, 1322, 307-315.
Schäfer RB, Kefford BJ, Metzeling L, Liess M, Burgert S, Marchant R, Pettigrove V, Goonan P, Nugegoda D. 2011. A trait database of stream invertebrates for the ecological risk assessment of single and combined effects of salinity and pesticides in South-East Australia. Science of the Total Environment, 40911, 2055-2063.
Schuijt LM, Peng FJ, Berg SJ, Dingemans MML, Van den Brink PJ. 2021. Ecotoxicological tests for assessing impacts of chemical stress to aquatic ecosystems: Facts, challenges, and future. Science of the Total Environment. 795. https://doi.org/10.1016/j.scitotenv.2021.148776
Sobrino-Figueroa A. 2015. Toxic effects of emerging pollutants in juveniles of the freshwater gastropod Physa acuta Draparnaud, 1805. American Malacological Bulletin, 332, 337-342.
Solé M, Shaw JP, Frickers PE, Readman JW, Hutchinson TH. 2010. Effects on feeding rate and biomarker responses of marine mussels experimentally exposed to propranolol and acetaminophen. Analytical and bioanalytical chemistry, 3962, 649-656.
Stockwell CA, Purcell KM, Collyer ML, Janovy J. 2011. Effects of salinity on Physa acuta, the intermediate host for the parasite Posthodiplostomum minimum: implications for the translocation of the protected White Sands Pupfish. Transactions of the American Fisheries Society, 1405, 1370-1374.
Suski JG, Salice CJ, Patiño R. 2012. Species-specific and transgenerational responses to increasing salinity in sympatric freshwater gastropods. Environmental Toxicology and Chemistry, 3111, 2517-2524.
Taylor D. 2015. The pharmaceutical industry and the future of drug development. Issues in Environmental Science and Technology, 1–33. https://doi.org/10.1039/9781782622345-00001
Tiwari M, Nagoji S, Kumar V, Tripathi S, Behera P. 2018. Oxygen isotope-salinity relation in an Arctic fjord Kongsfjorden: Implications to hydrographic variability. Geoscience Frontiers, 96, 1937-1943.
Trappe TA, Carroll CC, Dickinson JM, LeMoine JK, Haus JM, Sullivan BE, Lee JD, Jemiolo B, Weinheimer EM, Hollon CJ. 2011. Influence of acetaminophen and ibuprofen on skeletal muscle adaptations to resistance exercise in older adults. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 300(3), pp.R655-R662.
Velasco J, Gutiérrez-Cánovas C, Botella-Cruz M, Sánchez-Fernández D, Arribas P, Carbonell JA, Millán A, Pallarés S. 2019. Effects of salinity changes on aquatic organisms in a multiple stressor context. Philosophical Transactions of the Royal Society B, 374(1764), p.20180011.
Völker C, Gräf T, Schneider I, Oetken M, Oehlmann J. 2014. Combined effects of silver nanoparticles and 17α-ethinylestradiol on the freshwater mudsnail Potamopyrgus antipodarum. Environmental Science and Pollution Research, 2118, 10661-10670.
Wilcox JD, Bahr JM, Hedman CJ, Hemming JD, Barman MA, Bradbury KR. 2009. Removal of organic wastewater contaminants in septic systems using advanced treatment technologies. Journal of environmental quality, 381, 149-156.
Wilkinson JL, Boxall AB, Kolpin DW, Leung KM, Lai RW, Galbán-Malagón C, Adell AD, Mondon J, Metian M, Marchant RA, Bouzas-Monroy A. 2022. Pharmaceutical pollution of the world's rivers. Proceedings of the National Academy of Sciences. 1198. https://doi.org/10.1073/pnas.2113947119
Yang X, Chen F, Meng F, Xie Y, Chen H, Young K, Luo W, Ye T, Fu W. 2013. Occurrence and fate of PPCPs and correlations with water quality parameters in urban riverine waters of the Pearl River Delta, South China. Environmental Science and Pollution Research, 20(8), pp.5864-5875.
Yao B, Yan S, Lian L, Yang X, Wan C, Dong H, Song W. 2018. Occurrence and indicators of pharmaceuticals in Chinese streams: a nationwide study. Environmental Pollution, 236, 889-898.
Yu Y, Wu L, Chang AC. 2013. Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants. Science of the Total Environment, 442, 310-316.
Zukowski S, Walker KF. 2009. Freshwater snails in competition: alien Physa acuta Physidae and native Glyptophysa gibbosa Planorbidae in the River Murray, South Australia. Marine and Freshwater Research, 6010, 999-1005.
Zur J, Piński A, Marchlewicz A, Hupert-Kocurek K, Wojcieszyńska D, Guzik U. 2018. Organic micropollutants paracetamol and ibuprofen-toxicity, biodegradation, and genetic background of their utilization by bacteria. Environmental science and pollution research international, 2522: 21498–21524. https://doi.org/10.1007/s11356-018-2517-x