Do Wastewater Effluents Attract Aquatic Insects?

Assessing Behavioural Preference in Boatman Bugs (*Corixidae*) and Damselfly Larvae (*Zygoptera*)

Malin Hylland

Bachelor's thesis • 15 credits
Swedish University of Agricultural Sciences, SLU
Department of Applied Animal Science and Welfare
Ethology and Animal Welfare (BSc)
Uppsala 2025

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Supervisor:	Erin McCallum, Swedish University of Agricultural Sciences, Department of Wildlife, Fish and Environmental Studies
Examiner:	Maria Andersson, Swedish University of Agricultural Sciences, Department of Applied Animal Science and Welfare

Credits:	15 credits
Level:	First cycle, G2E
Course title:	Independent project in Biology
Course code:	EX0867
Programme/education:	Ethology and Animal Welfare (BSc)
Course coordinating dept:	Department of Applied Animal Science and Welfare
Place of publication:	Uppsala, Sweden
Year of publication:	2025
Cover picture:	Nele Otto
Copyright:	All featured images are used with permission from the copyright owner.
Keywords:	aquatic ecosystems, aquatic insects, behavioural ecology, behavioural preference, behavioural responses, contaminants, ecological traps, habitat preference, wastewater effluents, wastewater treatment plants

Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science

Department of Applied Animal Science and Welfare

Abstract

Ecological traps occur when organisms select habitats that appear suitable but are ultimately detrimental to their health and survival. Wastewater treatment plants (WWTPs) could contribute to the occurrence of ecological traps by emitting harmful pollutants into aquatic ecosystems where organisms live, potentially altering the behaviour and habitat preferences of aquatic species. This study examined the behavioural responses of boatman bugs (*Corixidae*) and damselfly larvae (*Zygoptera*) to treated WWTP effluent in a two-choice cue experiment. The experiment was conducted in a laboratory setting over two trials where the insects were exposed to cues from treated wastewater and clean tap water. Their behaviour was analysed using video tracking software to assess habitat choice, proximity to wastewater outlets, and activity levels. In trial 1, boatman bugs showed a significant avoidance towards the wastewater and the damselfly larvae showed a weak avoidance. Here, the median of the damselfly larvae was only slightly below 0.5 in the first trial, which could also potentially be read as indifference. In trial 2, both insects exhibited a significant preference for the wastewater. The fact that both species spent more time in the wastewater zone in trial 2 suggests possible habituation or attraction. These findings support the idea of WWTPs playing a role in the occurrence of ecological traps, highlighting the need for improved wastewater treatment and further research on the effects of contaminants on aquatic insects. Understanding these dynamics is crucial for creating sustainable management practices to conserve aquatic ecosystems.

Keywords: aquatic ecosystems, aquatic insects, behavioural ecology, behavioural preference, behavioural responses, contaminants, ecological traps, habitat preference, wastewater effluents, wastewater treatment plants

Table of contents

List of tables

List of figures

Abbreviations

CEC	Contaminant of Emerging Concern
LMM	Linear Mixed Model
SLU	Swedish University of Agricultural Sciences
TW	Tap Water
WW	Wastewater
WWTP	Wastewater Treatment Plant

1. Introduction

1.1 Ecological traps

Ecological traps occur when organisms choose to live in a habitat even though it is not optimal for their reproductive success and survival (Donovan & Iii, 2001). This could potentially lead to a reduction and, in the worst case, the extinction of a local population (Battin, 2004). One example of this is when species of aquatic insects, e.g., dragonflies (*Odonata)* and water bugs (*Hemiptera*), are drawn to surfaces which reflect polarized light, resembling the radiance of bodies of water typically used as feeding and breeding sites (Bernáth *et al*., 2002; Horváth *et al*., 2009; Heinloth *et al*. 2018). It's important to study the phenomenon of ecological traps because more knowledge about this subject could be a potential asset in future conservation work (Battin, 2004). The author emphasizes the need to focus on areas where ecological traps are most likely to occur and identify species most vulnerable to being trapped.

To be able to predict changes in aquatic ecosystems it's important to understand the different behaviours of its inhabitants (Cooke *et al*., 2013). The same authors point out that studying aquatic animals comes with its own unique set of challenges, where habitat complexity and the characteristics of a species, like small size and high mobility, can make tracking their behaviour in the wild difficult. This might be reflected in the currently limited amount of research on ecological traps in aquatic systems. According to Hale and Swearer (2016), ecological traps have mainly been studied in terrestrial ecosystems, particularly in relation to bird life, while relatively few studies have focused on freshwater ecosystems and their species. There could be many reasons why ecological traps occur in aquatic ecosystems, and despite the general lack of research, pollution has been shown to be one of them (Vonesh & Kraus, 2009).

1.2 Wastewater treatment plant effluents

Wastewater treatment plant (WTTP) effluents are a major source of pollution to aquatic environments and can contain a wide range of different compounds and substances (Adeleye *et al*., 2022). WWTP effluents can, for example, affect nearby bodies of water by releasing nutrients, increasing the amount of nitrogen (N) and phosphorus (P) and may spur eutrophication (Wurtsbaugh *et al*., 2019). This can lead to disturbances in aquatic ecosystems, including changes in the composition of aquatic food webs which in turn can cause a decline in biodiversity (Smith *et al*., 2006; Cai *et al*., 2013). Wastewater effluents can also contain contaminants of emerging concern (CECs) (Salimi *et al*., 2017). “CECs” is one of the collective names for an array of anthropogenic and natural compounds and substances, including microplastics, pesticides and pharmaceuticals (Edo *et al*., 2020; Rousis *et al*., 2021; Adeleye *et al*., 2022). Currently, conventional WWTPs can’t effectively eliminate all CECs, and therefore they continuously release pollutants in nearby rivers and streams (Gogoi *et al*., 2018). These pollutants have been shown to have negative impacts on aquatic animals, which in turn can threaten the aquatic ecosystems they are a part of (Saaristo *et al*., 2018; Rapp-Wright *et al*., 2023).

1.3 Pollution and its effect on wildlife

In a study by McCallum *et al*. (2019) it was discovered that fish communities looked different when comparing locations close to, and far away from, WWTP outfalls, where fish abundances were higher in locations close to outfalls. There are different theories as to why this may happen. Mehdi *et al*. (2021) bring up the factor of temperature, since their study showed that fish abundances close to WWTP outfalls increased, especially in winter, when the surrounding areas were colder and less optimal. Nutrients released from WWTPs can be another factor as to why fish are attracted to wastewater outfalls since it has been shown that the biomass of some species of fish has increased in wastewater enriched rivers (Askey *et al*., 2007; McCallum *et al*., 2019). So which consequences could come from aquatic animals showing attraction to WTTP outfall sites? In a study by McCallum *et al*. (2017) it was shown that pharmaceuticals from wastewater effluent can be stored in the body tissues of fish, as well as affect their behaviour. An example of this is fluoxetine, a selective serotonin reuptake inhibitor antidepressant drug, which have been shown to disrupt their reproductive behaviour (Wiles *et al*., 2020).

Apart from different species of fish, it has also been shown that contaminants from WWTP outfalls can have a negative impact on aquatic insects (Kraus, 2019; Previšić *et al*., 2020; Let *et al*., 2022). For example, endocrine-disruptive chemicals may heighten the stress response in caddisfly larvae (*Hydropsyche sp*.) (Previšić *et al*., 2020), and in a study by Späth *et al*. (2022) damselfly larvae exposed to wastewater effluents displayed a decrease in activity and foraging. For aquatic animals, contaminants may also affect the ability to perceive natural chemical cues (Scott & Sloman, 2004; Gross, 2022). This disruption can threaten key functions like foraging, reproduction, and predator avoidance, which largely rely on chemosensory input (Gross, 2022). For instance, damselfly larvae and water fleas (*Daphnia magna)* depend on chemical cues to detect predators, highlighting the potential ecological consequences of such impairments (Chivers *et al*., 1996; Beklioglu *et al*., 2010). While not as common, contaminants could also potentially affect the way cues are emitted, either by altering the structure of the cue, or by affecting the host emitting the cue (Ward *et al*., 2007). Diesbourg *et al*. (2025) higlights the importance of continually studying the functional responses of aquatic insects exposed to effluents, in order to better understand the risks to receiving ecosystems.

2. Aim of the study

Due to the limited amount of research on the effects of CECs and wastewater effluents on aquatic insects, the overall aim of this study is to gain knowledge about the potential role WWTPs play in the occurrence of ecological traps. Specifically, this study aims to test whether boatman bugs (*Corixidae*) and damselfly larvae (*Zygoptera*) show a preference for, avoidance of, or indifference towards wastewater effluent released from a WWTP. Ecosystem functions rely on the organisms within them maintaining and performing key behaviours (Schmitz *et al*., 2008). Since CECs can be harmful to aquatic insects, if populations remain in contaminated areas despite this, it could lead to the occurence of ecological traps and, in turn, the disruption of aquatic ecosystems. Additionally, it is important to note that also terrestrial ecosystems can be harmed by wastewater effluents, primarily through bioaccumulating insects being consumed by predators such as birds (Previšić *et al*., 2021). Gaining more knowledge about the effects of WWTPs is therefore crucial, as it could support the development of sustainable conservation management plans for aquatic environments and possibly help protect terrestrial ecosystems in the long run.

Boatman bugs, hereafter called “boatmen”, and damselfly larvae are two common aquatic invertebrates in northern Sweden. This study analysed videos of a two-choice cue experiment in a laboratory setting to test two primary hypotheses. The first hypothesis proposed that at least one of the tested insect species would show a significant preference for treated wastewater. The second hypothesis suggested that at least one of the species would show species-specific behaviour, especially physical activity, even under altered environmental conditions. In general, boatmen are highly mobile (Nowińska *et al*., 2023), while damselfly larvae tend to remain stationary for longer periods of time (Johnson, 1991). Consequently, the boatmen were expected to exhibit more movement compared to the damselfly larvae.

3. Materials and Methods

The data presented here are part of a larger research project investigating the behavioural preference of five different aquatic insects for wastewater effluents. My thesis work focused exclusively on the behavioural analyses of two of these five species. I have provided details on how the organisms were collected and their behaviours were recorded for completeness (Section 3.1 below, based on Rossi 2024), with my methodology beginning on Section 3.2, Video Analysis.

3.1 Behavioural trials and video recordings

The behavioural trials and videos were recorded in July 2023. No permits were needed for the collection of wastewater effluent or the collection of aquatic invertebrates used for the experiments.

3.1.1 Collection of wastewater

Treated wastewater effluent was collected from Umeå Wastewater Treatment Plant, and all the wastewater effluent used in this experiment was collected on the same day. At this treatment plant, wastewater undergoes mechanical, chemical, and biological treatment before being discharged into the environment (Vakin, 2023). The wastewater collected for the study was final treated effluent, meaning it had passed through all these three stages and was about to be discharged into a river called Umeälven .

Immediately after collection, the samples of wastewater were transported to the Swedish University of Agricultural Sciences, Umeå, where they were stored at -18°C. To minimize chemical changes in the wastewater, the samples were stored in the dark or kept covered. Every day, at the end of the experiment, a sample of wastewater was collected to measure concentration of a range of pharmaceutical pollutants. All compounds discovered, and their concentrations, were previously published in Rossi (2024) and can be found in the supplementary material (Appendix 1).

3.1.2 Collection of aquatic insects

The aquatic insects, hereafter "insects", were collected from Nydalasjön in Umeå, Sweden (63°49'26.058", 20°19'59.8974"). Fine-meshed nets on long handles, called sweep nets, were used to sweep through the aquatic vegetation to collect larvae of damselflies (*Zygoptera*) (n=30) and adult water boatmen (*Corixidae*) (n=30). The insects were then transported in a cooler to the laboratory at the Swedish University of Agricultural Sciences, Umeå. There they were placed in a climate-controlled room at 16°C to match outdoor temperature and to prevent further metamorphosis. The insects were then allowed to acclimatize to this temperature for 24 hours and after this, they were transferred to their holding tanks (50L glass aquariums), where each species was kept separately. The aquariums were filled with aged and aerated tap water and equipped with aquarium gravel, plastic zip ties and wooden popsicle sticks (mimicking woody aquatic vegetation) to provide refuges and substrate for the insects. This enrichment was provided because these insects prefer to "perch" or hold on to woody vegetation in the aquatic environment. It therefore aims to reduce stress and aggressive behaviour among the individuals. The specimens were collected two days before the first round of testing to ensure that their time spent in captivity was limited.

Boatmen were fed smaller damselfly larvae (a natural prey item, collected in addition to the experimental insects) and damselfly larvae were fed live zooplankton and thawed algae cubes (Akvarie Teknik, "Malawi Mix"). Stable conditions were maintained throughout the acclimatization period, as well as during the experiments (water temperature: [mean ± (SD)] 15 °C (± 0.9), oxygen saturation: 95.2% (± 2.4%), light:dark regime of 12:12 h).

3.1.3 Behavioural trials

Twelve glass aquariums, each with a capacity of 50 liters, were used as testing tanks in the experiments. To prevent the individuals from seeing each other when the tanks were placed next to each other during trials, the walls of the aquariums were covered with white adhesive plastic. The tanks were filled with white gravel (~1 cm) and 7 liters of aged tap water. On each short side of the aquarium, PVC pipes with a diameter of 1 cm were installed to hold hoses connected to peristaltic pumps. The pumps delivered the cues, which were either wastewater or tap water, during the trials (Figure 1).

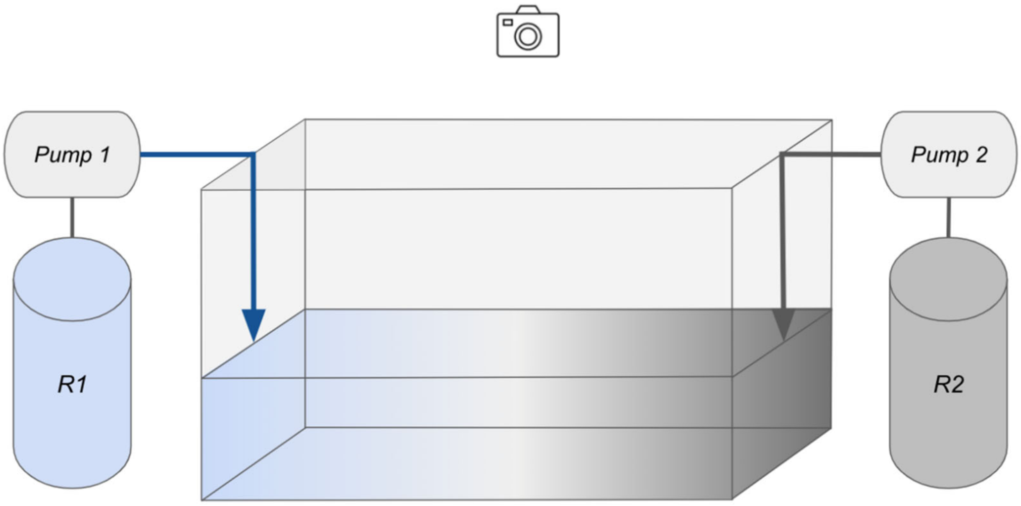

Figure 1. Schematic of the experimental setup, which included two peristaltic pumps (Pump 1 and Pump 2), two reservoirs for tap water and wastewater effluent (R1 and R2), an above-mounted camera (GoPro) and a glass aquarium.

Habitat preference trials consisted of a 20 min test period (5 minutes for adaptation and 15 minutes of free movement). First, the insect was transferred from its holding tank to a holding tube located in the center of the testing tank. The holding tube was made out of white PVC, 8 cm diameter and had drilled holes for water exchange with the surrounding environment. The pumps were activated, and the camera positioned above the tank started to record, when the test subject was placed in the holding tube. The animal remained in the tube for 5 minutes to acclimate before being released into the test tank by lifting the tube.

Throughout the trials, wastewater and aged tap water were supplied from opposite sides of the tank at a constant rate of 6 ml/min using peristaltic pumps. This flow rate was chosen based on previous food dye tests, which determined the optimal rate for the pumps by observing how long it took for the signals to meet in the middle of the tank. At 6 ml/min, the signals met in the middle after approximately ten minutes, hence the selected test duration of 15 minutes with a 5-minute adaptation period. The behavioural trials were recorded using GoPro Hero 8 cameras.

Each animal went through two trials during the experiment (“trial 1” and “trial 2”). For each trial, all the insects of each species were tested on the same day, with six insects being tested in six aquariums at the same time. Between trials, the tanks were cleaned by rinsing them with tap water and wiping them with 70% ethanol to eliminate any chemical cue residues. After a two-day interval, the trials were repeated for all individuals. The side where the wastewater effluent was presented during the trials were switched between the first and the second trial, and the side the cue was presented on was also counterbalanced within each trial so that half received the wastewater cue coming from the "right" side of the experimental room, while half received the wastewater cue coming from the "left" side of the experimental room. In between trials, insects were kept individually in plastic containers filled with water from their respective housing tanks to ensure individual identification during the second trial.

3.1.4 Wastewater pollutants and water quality measurements

Two samples from the wastewater effluent used in the trials, as well as a sample of the tap water used in the holding tanks and during the trials, were saved for later pollutant analyses. The methods for preparing and analysing these samples via liquid chromatography tandem mass spectrometry (LC-MS/MS) were not the focus of this thesis and are detailed in the study by Rossi (2024). The results can be seen in Appendix 1. Likewise, weekly water quality analyses were also made for each holding tank, the aged tap water, and the wastewater effluent (Rossi, 2024). General hardness GH/TH, carbonate hardness KH (mg/L $CaCO_3$), nitrite NO_2 (mg/L), nitrate NO_3 (mg/L), chlorine Cl_2 (mg/L) and pH were measured with the eSHa Aqua quick test. Conductivity (µS), TDS (ppm), salinity (ppt), as well as temperature (°C) were measured with the Hach Pocket Pro+ Multi 2 Tester. Dissolved oxygen DO (mg/L and %) was measured with YSI Ecosense ODO200 Optical Dissolved Oxygen Meter.

3.2 Video analysis

Video analyses were conducted between March-May, 2024. A total of 117 videos, consisting of 59 videos of boatmen and 58 videos of damselfly larvae, were tracked and analysed in EthovisionXT (version 16.0.1538, 2021), a software used for tracking and analysing the behaviour of animals in laboratory settings. The software identifies the x and y position of the tested individuals during trials.

Specifically, in EthovisionXT each testing tank in the video recordings was divided into two zones to facilitate later behavioural analysis: zone 1 and zone 2 (Figure 2). In the first trial, zone 1 was the "wastewater zone" (WW zone) and zone 2 was the "tap water zone" (TW zone) (Figure 2). In the first trial, zone 1 was the WW zone and zone 2 was the TW zone. Two points in the videos were identified as the wastewater outlet (WW outlet) and the tap water outlet (TW outlet) to facilitate later analysis. After the zones and points of interest were identified, detection settings were made for each video. This involved choosing a sample frame rate for each video. We selected as close to "20" as possible (all videos had a frame rate of 20, except 5 that were 19.67 – this was set by the software on the camera and was not editable). The making of the detection settings also involved manually outlining each individual to let the software know what shape it was supposed to track. To further facilitate the tracking process, the detection settings were set to “darker” for “subject color compared to background” and “video pixel smoothing” was set to “high”.

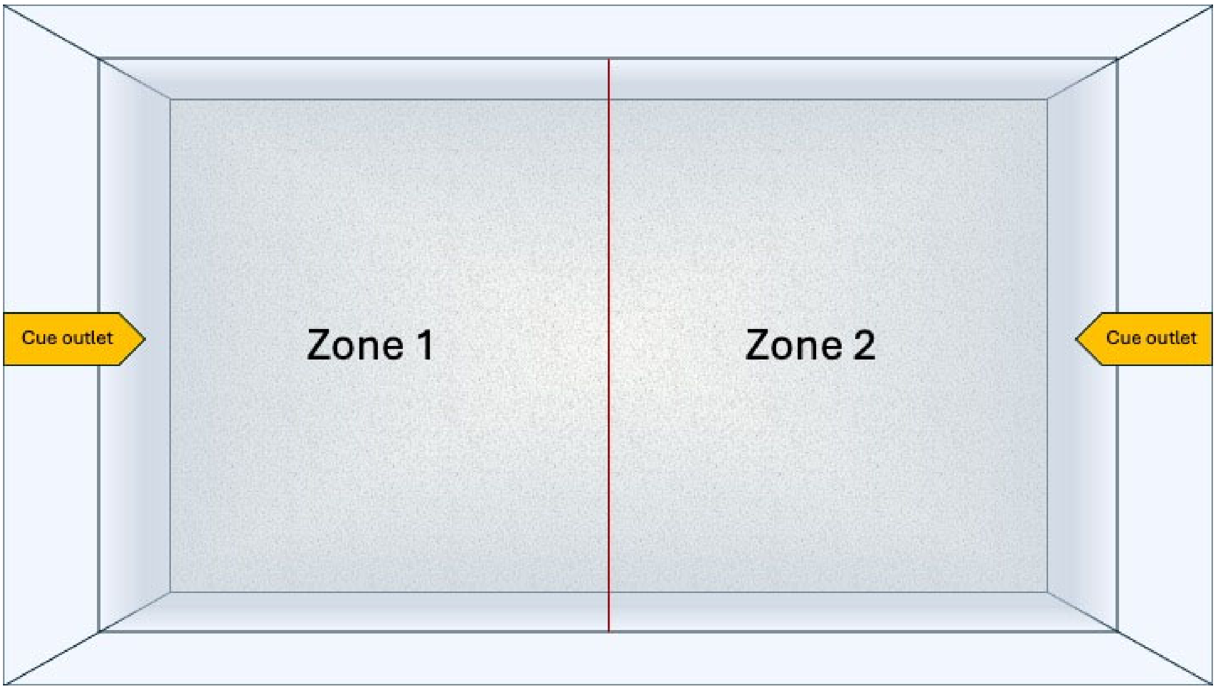

Figure 2. Schematic over how the video recordings were digitally divided into “zone 1” and “zone 2” for further analyses.

After detection settings were customized for each video, the videos were acquired. Although EthovisionXT is a fully automated program, errors in tracking still occur when the program cannot detect the animal. These errors were solved by manually going through each video to correct false detections. This process involved looking for sharp, unnatural angles in the tracking pattern. To correct instances where the software lost the insect, missing samples were interpolated. This made sure all the incorrect data points were removed and replaced with the correct data. Examples of two videos from the trials with corrected data can be seen in Figure 3.

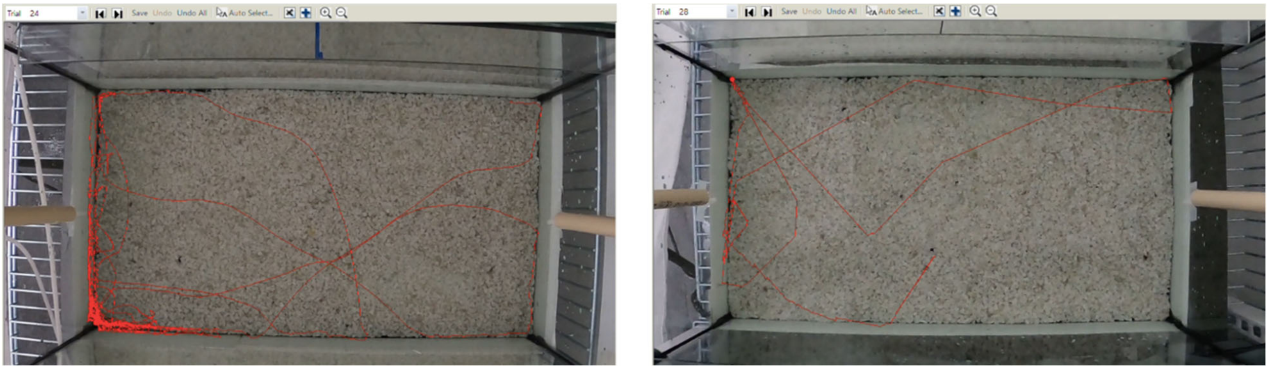

Figure 3. Stills from two different videos with corrected data showing the movement of one individual, visualized with a red line in EthovisionXT. From left to right: Boatman bug and damselfly larvae.

When the tracking data had been corrected, the tracking software calculated several behavioural metrics used in my analyses including: the duration (sec) for which the animal spent in each selected zone (zone 1 and zone 2), distance (cm) to point (WW outlet and TW outlet) and total distance (cm) moved between species and trials. The data from EthovisionXT was exported as excel file and merged to create a complete dataset for further analyses in R (version 4.2.2, 2022-10-31).

3.3 Statistical analysis

All statistical analyses described were performed in R (version 4.2.2, 2022-10-31) using the basic default libraries, as well as the packages *tidyverse* 2.0.0, *DHARMa* 0.4.6, *performance* 0.11.0 and *glmmTMB* 1.1.9.

3.3.1 Hypothesis I

To test hypothesis I, I first ran two analyses that tested the proportion of time individuals spent in the wastewater zone of the aquarium. The first statistical test included all individuals in the test, while the second only included individuals which had sampled both sides of the testing tank. A third statistical test (Distance to WW outlet) was then done to compare the distance (cm) each insect kept to the wastewater outlet between species and trials. For all three analyses, interaction between species and trial was tested.

To analyse the proportion of time individuals spent in the WW zone of the aquarium, a histogram was made to inspect the distribution of the data. The Shapiro-Wilk test was applied to test the normality of the data distribution and plots of residual patterns against fitted values were used to inspect homogeneity of variance. Cumulative time was changed to proportion of the trial time and a generalized linear mixed-effects model with a beta distribution was fitted to the data because of its suitability for proportion data bounded between 0 and 1. The chosen fixed effects were "species" and "trial", and "animalID" was included as a random effect. Apart from the model's suitability for proportion data bounded between 0 and 1, the use of this model was imperative to account for repeated measures across trials and substantial variability across the 117 observations. To conclude, interaction between species and trial was tested.

The same type of model was applied a second time, but on a filtered dataset. When looking at the data, a slightly bimodal distribution was discovered, indicating a variability in behaviour among individuals, where some of the individuals only visited one zone during the full duration of a trial. Therefore, a second analysis was done with a filtered dataset, only including animals which visited both zones during the trials. This was done to restrict the analysis to individuals that were most likely to be making a choice between the two cues because they had visited both sides of the aquarium. The generalized linear mixed-effects model was re-fitted to the filtered data. Initially, the model included "animalID" as a random effect, but due to a reduction in sample size that led to model convergence failures, the random effect needed to be removed. Diagnostics were repeated for the new model without the random effect to ensure it still met assumptions. Again, interaction between species and trial was tested.

To compare the distance (cm) each insect kept to the wastewater outlet between species and trials, I first created a histogram to inspect the distribution of the data. The Shapiro-Wilk test was applied to test the normality of the data distribution and plots of residual patterns against fitted values were used to inspect homogeneity of variance. A linear mixed model was fitted to the data with "species" and "trial" as fixed effects and "animalID" as a random effect. The use of this model was, yet again, imperative to account for repeated measures across trials and substantial variability across the 117 observations. Also here, the dataset was tested for interaction between species and trial.

3.3.2 Hypothesis II

To test hypothesis II, a statistical test looking at the total distance moved (cm) between species and trials was conducted. As previously, a histogram was created to inspect the distribution of the data. The Shapiro-Wilk test was applied to test the normality of the data distribution and plots of residual patterns against fitted values were used to inspect homogeneity of variance. We observed that the assumption of homogeneity of variance was violated and that there was greater variance in the boatmen than in the damselfly data. To address this, a dispersion formula was applied by level of species to account for the heteroskedasticity, resulting in better model diagnostics. To test for the effect of species and trial on the total distance moved, a linear mixed-effects model was fitted to the data, with "species" and "trial" as fixed effects, "animalID" as a random effect, and the dispersion formula by species. As already mentioned, using this model was necessary to handle the repeated measures across trials and the substantial variability present in the 117 observations.

4. Results

4.1 Water samples

The results of the analysis of water samples, including tap water and two wastewater samples (wastewater 1 and wastewater 2) showed the concentration of various substances in nanograms per liter (ng/L) and can be found in Appendix 1 (originally published in Rossi, 2024). No pollutants were detected in the tap water control. Overall, 40 different pharmaceuticals of the total 71 that were screened for were detected. In general, there was high repeatability of the compounds detected and the concentrations measured between the two wastewater samples.

4.2 Behavioural trials

A total of three deaths were observed between trial 1 and trial 2 (one boatman bug and two damselfly larvae). Consequently, 60 videos were analysed from trial 1 and 57 from trial 2.

4.2.1 Hypothesis I

4.2.1.1 Time in WW zone, unfiltered dataset

The first analysis was conducted on the complete dataset including all recorded observations. It revealed a main effect of species (Figure 4; GLMM, N=117, [estimate ± s.e.] 0.86 ±0.33, $Z = 2.60$, $P = 0.0092$) and a main effect of trial (Figure 5; GLMM, N=117, [estimate ± s.e.] 1.02 ± 0.32, $Z = 3.21$, $P = 0.0013$) on the proportion of time insects spent in the WW zone. Damselfly larvae spent more time in the WW zone compared to the boatmen and both species spent more time in the WW zone in the second trial compared to the first trial.

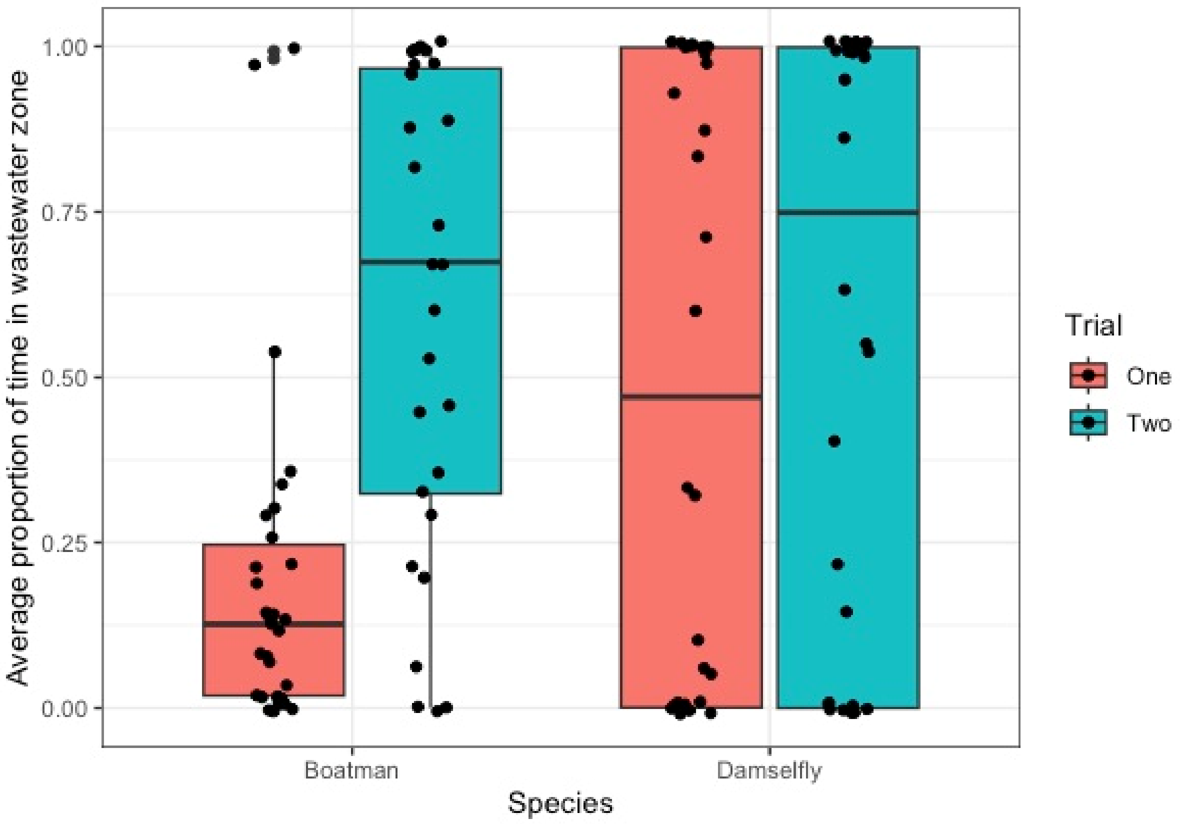

Figure 4. Box plots showing medians and inter-quartile range of time spent in the wastewater zone between species and trials (complete dataset).

Mean values, standard deviations and total observations for the complete dataset can be seen in Table 1.

Table 1. Mean, standard deviation (SD) and number of observations (N) for proportion of time spent in WW zone (complete dataset)

Trial	Species	Mean	SD	N
One	Boatman	0,1925	0,2527	30
One	Damselfly	0,4929	0,4564	30
Two	Boatman	0,6211	0,3619	29
Two	Damselfly	0,5805	0,4451	28

4.2.1.2 Time in WW zone, filtered dataset

For boatmen, the filtered dataset included N=28 (trial 1) and N=23 (trial 2), and for damselfly larvae, the filtered dataset included N=14 (trial 1) and N=10 (trial 2). The analysis of the filtered dataset revealed a non-statistically significant trend for an effect of species (Figure 5; GLMM, N=75, [estimate ± s.e.] 0.77±0.4, Z = 1.93, P = 0.054) and a main effect of trial on the proportion of time insects spent on the wastewater side (Figure 5; GLMM, N=75, [estimate ± s.e.] 1.25±0.35, Z = 3.55, P = 0.00038). There were small differences between the two species regarding how much time they spent in the WW zone. In trial 1, damselfly larvae spent more time in the WW zone compared to the boatmen, and in trial 2, the opposite was true. Both species increased their time spent in the WW zone between the first and the second trial.

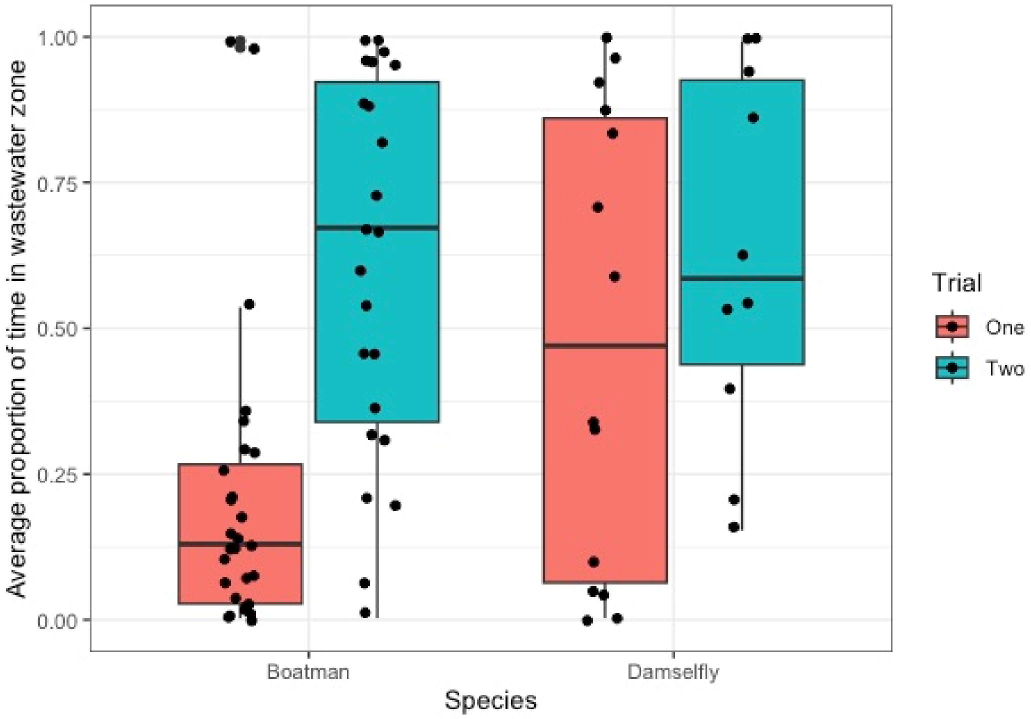

Figure 5. Box plots showing medians and inter-quartile range of time spent in the wastewater zone between species and trials (filtered dataset).

Mean values, standard deviations and total observations for the filtered dataset can be seen in Table 2.

Table 2. Mean, standard deviation (SD) and number of observations (N) for proportion of time spent in WW zone (filtered dataset)

Trial	Species	Mean	SD	N
One	Boatman	0,2062	0,2563	28
One	Damselfly	0,4856	0,3971	14
Two	Boatman	0,6094	0,3249	23
Two	Damselfly	0,6268	0,3132	10

4.2.1.3 Distance to cue source point

The analysis testing the average distance to WW outlet between species and trials revealed a significant interaction between species and trial (Figure 6; LMM, N = 117, [estimate ± s.e.] 11.96 ± 3.41, Z = 3.50, $p < 0.001$). The interaction revealed that boatmen swam closer to the wastewater outlet in trial 2 compared to trial 1, while the damselfly larvae showed no significant change in their behaviour between the two trials.

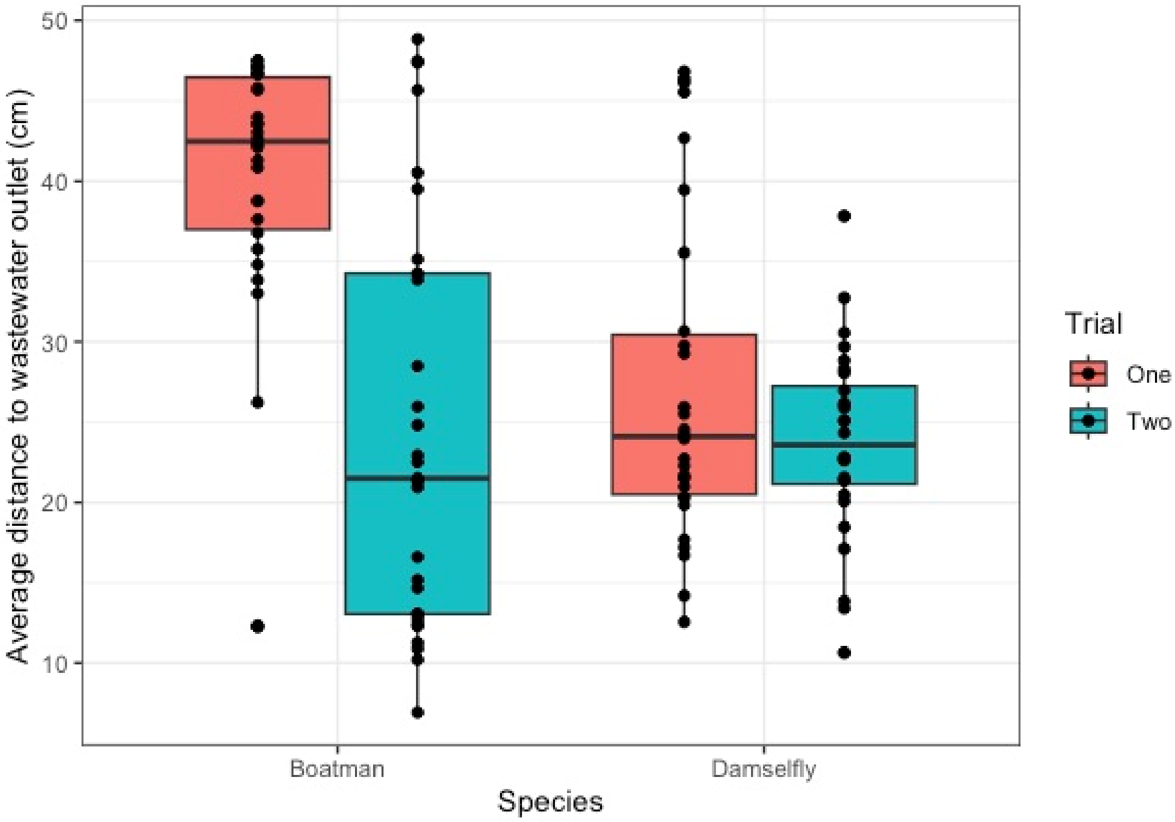

Figure 6. Box plots and inter-quartile ranges showing median distance to wastewater outlet between species and trials (complete dataset).

Mean values, standard deviations and total observations for average distance to wastewater outlet can be seen in Table 3.

Table 3. Mean in centimeters, standard deviation (SD) and number of observations (N) for average distance to WW outlet (complete datset)

Trial	Species	Mean	SD	N
One	Boatman	39,71	9,11	30
One	Damselfly	27,06	10,12	30
Two	Boatman	24,5	13,01	29
Two	Damselfly	23,74	5,93	28

4.2.2 Hypothesis II

4.2.2.1 Total distance moved

A significant interaction was found between species and trial on the total distance moved

(Figure 7; LMM, N = 117, [estimate ± s.e.] 273.25 ± 118.93, Z = 2.30, P = 0.022) where both insects appeared to move less in the second trial, but this was more pronounced for the boatmen relative to the damselfly larvae. In general, boatmen travelled a longer distance in both trials compared to the damselfly larvae (Table 3).

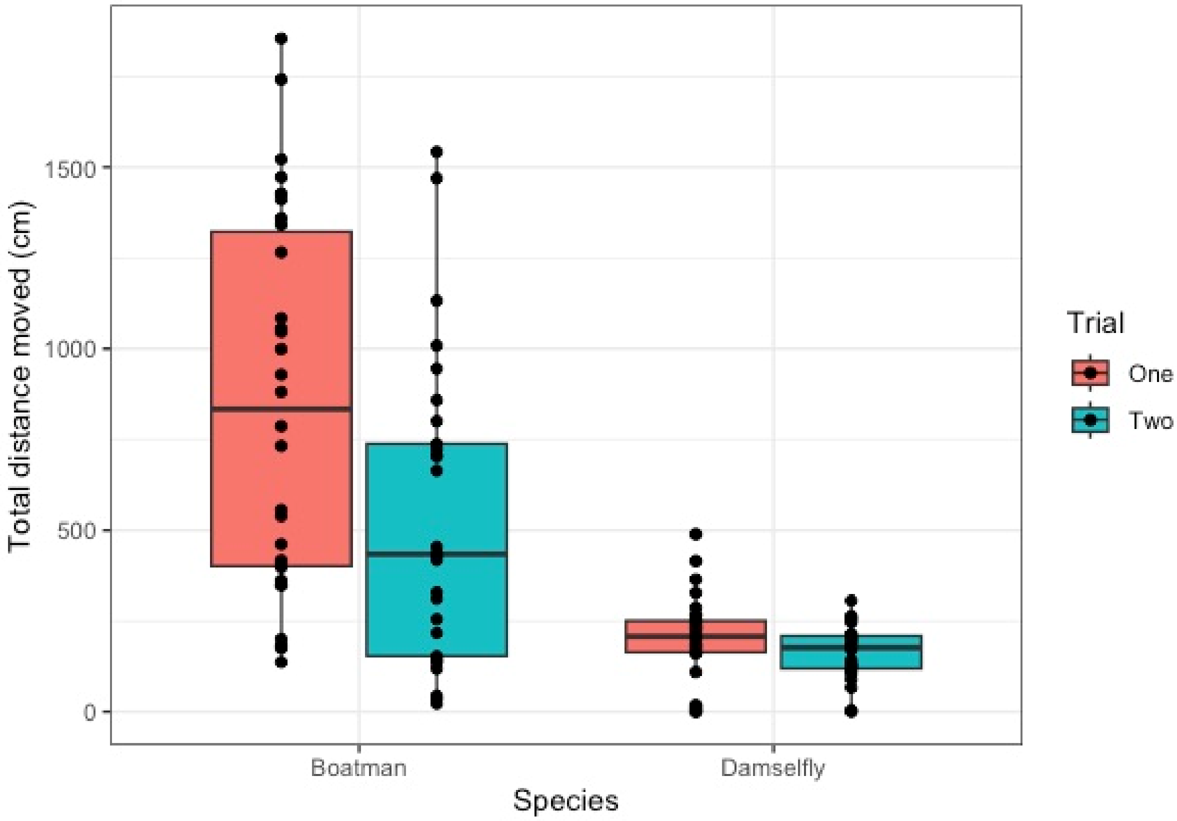

Figure 7. Box plots and inter-quartile ranges showing median distance moved between species and trials.

Mean values, standard deviations and total observations for total distance moved can be seen in Table 4.

Table 4. Mean in centimeters, standard deviation (SD) and number of observations (N) for total distance moved

Trial	Species	Mean	SD	N
One	Boatman	848.26	513.25	30
One	Damselfly	201.42	117.04	30
Two	Boatman	530,15	415.12	29
Two	Damselfly	159.58	79.94	28

5. Discussion

5.1 Water samples

The analysed samples of the treated wastewater contained several pharmaceuticals and illicit drugs (Appendix 1). The study by Rossi (2024) brings up concerns regarding a few different substances found in the water samples with notably high concentrations, for example fluconazole (antifungal), diclofenac (anti-inflammatory) and metformin (antidiabetic). The effects of these substances on aquatic organisms seem to differ in type and severity. For fish, fluconazole can for example lead to embryo development issues (Escobar-Huerfano *et al*., 2022), diclofenac can reduce performance behaviour (Nassef *et al*., 2010) and metformin can cause oxidative stress (Sibiya *et al*. 2023). In general, information regarding how specific substances in wastewater affect boatmen and damselfly larvae is lacking, however there is considerable more studies focusing on damselfly larvae, or other species of the order *Odonata*, compared to boatmen. This difference might be explained by the fact that damselfly larvae are known to be sensitive to environmental changes, potentially making them useful indicators of water quality (Van Praet et al. 2014) and therefore valuable subjects in environmental research.

Looking at how the different compounds found in the water sample analysis can affect species of dragonfly *(Odonata)*, (Jonsson *et al*., 2019) showed one example where the antihistamine diphenexample seemed to worsen damselfly larvae's ability to swim, indicating a reduced ability to evade predators. And even though only small amounts of diphenexample showed up in the treated wastewater analysis of this study, the same authors proclaim that small amounts can be enough to have an effect. Another example can be found in a study by Bláha *et al*. (2019), where citalopram and tramadol, when presented individually, decreased the feeding rate of the blue hawker (*Aeshna cyanea*). However, the same authors also showed that an increase in feeding rate happened when the individuals were exposed to treated wastewater containing a variety of compounds, emphasizing how different the effects can look when compounds are mixed. This was also highlighted in another study which indicated that diclofenac could cause oxidative stress and affect gene expression related to detoxification processes in harlequin fly larvae (*Chironomus riparius*), especially when also mixed with cadmium (Xie *et al*., 2020).

Another thing important to note is that some substances seem to have different effects on different species. For example, the antibiotic drug sulfamethoxazole, which was one of the substances in this study with notably high concentrations, has been shown to be beneficial to flatworms (*Mesostoma*) but detrimental to dragonfly larvae (*Odonata*) (Schuijt *et al*., 2024). The same authors therefore emphasize the importance of studying the effects of contaminants on several trophic levels to be able to assess their overall impact.

5.2 Behavioural trials

Hypothesis I predicted that at least one of the tested species would show a significant preference for treated wastewater. In hypothesis II, it was predicted that at least one of the species would continue to show species-specific behaviours, particularly physical activity, even under altered environmental conditions. Hypothesis I tested for average time spent in WW zone and average distance to WW outlet, while hypothesis II tested for total distance moved. Below, I will discuss the responses of each species, in turn.

5.2.1 Boatman bugs

When testing hypothesis I, the boatmen showed clear preferences; avoidance towards the wastewater in trial 1 and preference for the wastewater in trial 2. This was further affirmed when analysing the filtered data set, since the results for both datasets showed only minor differences. As mentioned, the filtered dataset only included animals which sampled both zones during the trials, and both sets were analysed to increase the chance of habitat choice being based on actual habitat preference, rather than by external factors. Their preference changing between trials was an unexpected find, and this observed change can be interpreted in several ways. One explanation is habituation, where repeated exposure to the wastewater effluent reduced their initial avoidance response. Alternatively, this shift could indicate that a specific characteristic of the wastewater effluent becomes more attractive to the insects after repeated exposure.

When testing hypothesis II, the results came out as predicted, with boatmen showing considerably more movement than the damselfly larvae. The comparably high level of activity aligns with their natural behaviour since, in the wild, boatmen frequently move between the bottom and the surface to forage and breathe, and their legs are specifically adapted for swimming (Hadicke *et al*., 2017; Nowińska *et al*., 2023). According to Hadike *et al*. (2017), boatmen foraging generally occurs under active movement, which is another factor that could have contributed to result. However, a decrease in movement was seen between trials, where the boatmen moved less in trial 2. This might once again suggest environmental adaptation due to reduced stress after familiarization. The reduction in movement could also possibly be supporting the idea that the boatmen, after having sampled it once before, found a perceived benefit from staying in the wastewater zone, and in turn weren't as motivated to explore the other side of the testing tank.

5.2.2 Damselfly larvae

The damselfly larvae, in contrast to the boatmen, did not display a strong avoidance in trial 1, with a median only slightly below 0.5. This low number could suggest indifference towards the wastewater, rather than avoidance. In trial 2, however, although not as pronounced as in the boatmen, they also exhibited a preference for the WW zone. The general lack of avoidance between both trials could point to the damselfly larvae not being affected by, or not being able to distinguish between, the two cues. It could also possibly even be read as preference for the wastewater. When testing for average time spent in WW zone, in comparison with the boatmen, the damselfly larvae showed a smaller difference in results between trials, something which was further confirmed when looking at the average distance to WW outlet. For damselfly larvae, the mean for distance to WW outlet only differed by a little more than 3 cm between trials, whereas for boatmen, it differed by more than 15 cm. Even though the changes between trials were relatively small there were still changes to be seen, which could mean that they, just like the boatmen, exhibited signs of habituation.

As with the boatmen, it's important to account for natural behaviours when analysing the results. In the wild, as mentioned by Johnson (1991), the damselfly larvae spend long periods of time being stationary. This could also be seen in this experiment, where the damselfly larvae were frequently seen remaining still in the same spot. Not moving is one of the defense mechanisms in the damselfly larvae's repertoire of anti-predator behaviours (McPeek, 1990), which could mean that the choice of zone during the trials was influenced, more by their natural reaction to a perceived threat, than by a response to the cues. Even while foraging, the damselfly mostly sits still and waits for their prey to be in reach before they attack (McPeek & Crowley, 1987), which is yet another factor which could've contributed to the comparably low activity level of the damselfly larvae. While it can be challenging to differentiate between responses driven by natural behaviours and those caused by external factors, the general lack of avoidance towards the wastewater remains a noteworthy finding with important implications for environmental research.

5.3 Ecological implications

The findings of this study contribute to the growing amount of evidence that WWTP effluents can possibly play role in the occurence of ecological traps. Both species studied here showed a lack of consistant avoidance, and to some extent they even showed preference for the treated wastewater. Preferences changing between trials suggest potential habituation or attraction, which further increases the potential risk of them staying in contaminated areas. Since it has been shown that compounds found in WWTP effluents can have negative effects on aquatic insects, this behavioural response is concerning. The possible long-term threats of exposure to contaminants extend beyond individual survival, as aquatic insects are critical components of freshwater food webs and essential to ecosystem functioning (Schowalter, 2022). Their altered distribution and reduced fitness could therefore ultimately threaten biodiversity and ecosystem services (Harrison *et al*., 2010; Schowalter, 2022).

Furthermore, the findings might have implications for terrestrial ecosystems, since bioaccumulating insects may transfer these contaminants to terrestrial predators, such as birds, and thereby broadening the ecological impact of wastewater pollution (Previšić et al. 2021). This reinforces the importance of considering both aquatic and terrestrial ecosystems when assessing the environmental effects of WWTPs.

5.4 Limitations

5.4.1 Video analysis

Although EthovisionXT is a fully automated program, errors in tracking still occurred when the program was unable to detect the insects. This usually happened with small damselfly larvae or when the dark boatmen positioned themselves in the dark corners of the testing tanks. Poor video quality, disturbances on the water surface and reflecting light were other factors which contributed to the faulty tracking. These issues were fixed manually, which is part of the EthovisionEXT work flow. However, it was both time consuming and risked exposing the study to errors caused by the human factor.

5.4.2 Behavioural trials

To optimize the laboratory setting in the videos analysed in this study, I believe a couple of things could have been adjusted. For example, replacing the black sealant with a lighter one could potentially have facilitated the tracking software's detection of individuals. The black sealant may also have contributed to the fact that some insects spent most of their time standing still in corners, since the dark colour could've been interpreted as safe hiding spots. For damselfly larvae for example, areas without shelters are generally avoided and if not motivated, for example by the presence of prey, they may stay immobile as to not grab the attention of predators (Stoks, 1999).

That this study was conducted in a laboratory setting is important to keep in mind when analysing the results for the purpose of understanding real-life scenarios, since in a laboratory setting it might be impossible to create an environment with all the synergetic factors existing in situ. For example, the impact of a pollutant in freshwater can't be fully assessed by looking at only one factor, and the result will be biased if indirect effects are ignored (Gergs *et al.* 2013). Another factor to consider is the general lack of knowledge on how captivity affect the welfare and consequently the behaviour of insects. According to van Huis (2021), insect welfare hasn't been prioritized in science, probably due to the common belief that insects don't experience pain or stress. However, according to Crump *et al.* (2023), there are studies which points to this not being true, and Gjerris *et al.* (2016) emphasizes that "absence of proof should not be misunderstood as proof of absence" when it comes to insect welfare.

Because of the large biodiversity of insects, creating general insect welfare guidelines for insects in captivity might prove to be difficult, but maintaining an environment similar to their natural habitat is likely the most reliable way to ensure their well-being (De Goede *et al.*, 2013). In this study, in an attempt to mimic natural conditions and promote natural behaviours, the holding tanks were provided with gravel and hiding structures. However, fully replicating the insects' natural environment in a laboratory setting remains difficult. This raises the question of whether the observed decrease in activity levels between trials in this study might be partially explained by the effects of captivity, rather than solely by their adaption to the environment.

5.5 Future studies

A large portion of the existing research seems to focus on the impact of pesticides on different species of *Odonata* (Villalobos-Jimenez *et al*., 2016), with limited studies specifically addressing *Corixidae*. Since boatmen appear to be at risk of being attracted to wastewater, it is important to investigate the potential effects of this exposure. Furthermore, several of the pesticides examined in existing studies are now banned in Sweden, e.g., endosulfan, atrazine and chlorpyrifos *(Wallin et al*., 2012; Havs- och Vattenmyndigheten, 2014; Naturskyddsföreningen, 2021). While such research provides insight into the risks agricultural pollution poses to aquatic insects, it does not adequately address the present effects of treated wastewater on boatmen in Sweden specifically. Further research on how the different substances of Swedish WWTP effluents affect aquatic insects could therefore support the development of sustainable management plans for the conservation of Swedish aquatic ecosystems.

As previously noted, in-situ experiments could be helpful to better assess the real-world effects of WWTP effluents. Future research should also incorporate a wider range of aquatic insect species to improve our understanding of the synergistic processes that drive aquatic ecosystem functions. Including additional species might also help point out which ones are at most risk of ending up in ecological traps, possibly making it easier to take preventative measures to avoid decreases in local populations of insects. One factor that may be important to consider when planning and analysing future studies is that the individuals in this study exhibited different behaviours between trials conducted under similar conditions. This especially highlights the importance of including multiple trials in behavioural studies when wanting to draw reliable conclusions regarding behavioural responses.

Furthermore, continuous investigation of specific substances present in WWTP effluents could prove valuable to understand the effects they have on ecosystems, for example by focusing on how pollutants from wastewater accumulate in aquatic insects and affect their long-term survival. Also, while no current studies confirm whether aquatic insects are attracted to particular substances found in WWTP effluents, observed signs of attraction towards contaminated water suggest this might be the case. Testing for such attraction could therefore help identify which substances to focus on when developing more effective wastewater purification methods. Overall, because of the risk WWTP effluents may pose to the environment, regulation of CECs and water treatment method development should be prioritized.

5.6 Conclusion

This study investigated whether boatman bugs and damselfly larvae show signs of attraction towards WWTP effluents. This was done by conducting a two-cue test over two trials in a laboratory setting, exposing the insects to treated wastewater and tap water. When testing for preference for treated wastewater, in trial 1, boatmen showed significant avoidance, whereas the damselfly larvae showed only a minor avoidance. In fact, the damselfly larvae's result from trial 1 showed a median only slightly below 0.5, which could also possibly be read as indifference rather than avoidance. This changed in trial 2, where both species showed a preference for the WW zone, possibly indicating the insects' ability to habituate to the wastewater. The significant effect of trial however, was especially pronounced in boatman bugs and weaker in damselfly larvae. These findings contribute to a better understanding of how species and trial conditions influence the time aquatic insects spend in contaminated waters, emphasizing the need to consider both factors in environmental and behavioural studies. Although the tests were conducted in a laboratory setting, I believe these findings can be applied to the development of ecosystem management plans, provided potential errors have been taken under consideration. This statement is supported by the insects' apparent display of natural behaviours during the experiment.

The general lack of consistent avoidance of wastewater by the insects in this study suggests that they may remain in contaminated areas, even in the wild. This tendency raises concerns about WWTPs and their potential role in the occurence of ecological traps, which could ultimately threaten aquatic insects and the ecosystems they support. These findings highlight the need for stricter regulation of CECs and the development of advanced wastewater treatment methods, as well as the need for further research on how contaminants can affect aquatic animals.

6. Popular science summary

In nature, there’s a phenomenon called ecological traps, which happens when animals misjudge the quality of a habitat and mistake harmful habitats for safe ones. This can lead them to settle in areas that are harmful to their overall health and survival. If enough animals make the same mistake, it could even cause local populations to die out. These traps can occur both on land and in water, and they affect many types of animals, including insects. For example, flying insects may be attracted to the way light reflects off an oil spill because it reminds them of a pond or lake where they would normally lay their eggs. Of course, oil spills are anything but safe for insects, and definitely not a good place to lay eggs.

Treated wastewater, which is released into rivers and lakes by wastewater treatment plants, might also play a role in the occurrence of ecological traps. Even though this water has been treated, it often still contains pollutants like leftover medicines, pesticides, and microplastics. Previous studies have shown that long-term exposure to polluted water can harm aquatic animals, but surprisingly, these animals don’t always seem to avoid wastewater outfall sites. In fact, in some situations there has been signs pointing to fish showing attraction towards these areas, putting themselves at risk by exposing themselves to contaminants.

For fish, common contaminants in wastewater effluents have been shown to disrupt their ability to swim, reproduce, and communicate. While less is known about how pollution affects aquatic insects, evidence shows that some species of aquatic insects, for example the damselfly larvae, can be affected in similar ways. To explore this further, my study tested the behaviour of two aquatic insects, boatman bugs (*Corixidae*) and damselfly larvae (*Zygoptera*), to see if they would avoid, be attracted to, or show an indifference towards treated wastewater. Each insect was placed in an open tank divided into two zones containing one outlet each: one with clean tap water and the other with treated wastewater. The treated wastewater samples were collected from a wastewater treatment plant in Umeå, Sweden, and had undergone all cleaning processes, in theory ready to be released into the river Umeälven. When testing the wastewater samples, they came up positive for a wide range of different compounds of various amounts, for example antibiotics, antidepressants and anti-inflammatory drugs.

The insects were observed over two trials and in the first trial, boatman bugs showed a strong avoidance of the wastewater zone, and the damselfly showed a weak avoidance. In fact, the damselfly larvae’s signs of avoidance were so weak, the results could also possibly be read as indifference. In the second trial however, the behaviour of both species changed, where they both showed an attraction to the wastewater zone, a shift that was especially noticable in the boatman bugs. There are several possible explanations for this behaviour. One idea is that repeated exposure to the wastewater caused the insects to change their response, possibly becoming accustomed to it. Another theory is that there may be a specific characteristic in the wastewater that attracts them after they've been exposed to it once before. Regardless of the reason, the lack of avoidance in the second trial may point to the risk of insects staying in contaminated waters in the wild, consequently ending up in ecological traps. Since insects play important roles in aquatic ecosystems, this could result in the disruption of entire ecosystems, which would in turn reduce biodiversity and cause the loss of important ecosystem services.

This study highlights how wastewater treatment plants might contribute to the occurrence of ecological traps in aquatic environments. Understanding how and why this happens could help us protect these ecosystems. For example, knowing whether insects can detect pollutants and how their behaviour changes over time could help scientists predict when and where ecological traps might occur. To build on these findings, future research could test other insect species and study their behavioural responses. More research could also focus on how pollutants from wastewater accumulate in aquatic insects and affect their long-term survival. Such studies could be key in the creation of sustainable management plans for freshwater ecosystems, ensuring their long-term health and survival.

Acknowledgements

This thesis would not have been possible without the invaluable support and guidance I received along the way. First, I would like to sincerely thank my supervisor, Erin McCallum, for your patience, for sharing your expertise, and for creating an encouraging environment where I always felt comfortable asking for advice. I'm especially grateful for your flexibility and support, which allowed me to pursue a topic of great interest to me, even though we were based in different cities. I also want to thank my friends for their constant encouragement, moral support and well-timed ice cream breaks, all of which played a vital role in the making of this thesis. Finally, thank you to my classmates for your thoughtful feedback, insightful comments and critical questions, which contributed valuable perspectives to my work. Thank you to everyone who played a role in making this thesis possible.

References

Adeleye, A.S., Xue, J., Zhao, Y., Taylor, A.A., Zenobio, J.E., Sun, Y., Han, Z., Salawu, O.A. & Zhu, Y. (2022). Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. *Journal of Hazardous Materials*, 424, 127284. https://doi.org/10.1016/j.jhazmat.2021.127284

Askey, P.J., Hogberg, L.K., Post, J.R., Jackson, L.J., Rhodes, T. & Thompson, M.S. (2007). Spatial patterns in fish biomass and relative trophic level abundance in a wastewater enriched river. *Ecology of Freshwater Fish*, 16 (3), 343–353. https://doi.org/10.1111/j.1600-0633.2007.00221.x

Battin, J. (2004). When Good Animals Love Bad Habitats: Ecological Traps and the Conservation of Animal Populations. *Conservation Biology*, 18 (6), 1482–1491. https://doi.org/10.1111/j.1523-1739.2004.00417.x

Beklioglu, M., Banu Akkas, S., Elif Ozcan, H., Bezirci, G. & Togan, I. (2010). Effects of 4-nonylphenol, fish predation and food availability on survival and life history traits of Daphnia magna straus. *Ecotoxicology*, 19 (5), 901–910. https://doi.org/10.1007/s10646-010-0470-7

Bernáth, B., Szedenics, G., Wildermuth, H. & Horváth, G. (2002). How can dragonflies discern bright and dark waters from a distance? The degree of polarisation of reflected light as a possible cue for dragonfly habitat selection. *Freshwater Biology*, 47 (9), 1707–1719. https://doi.org/10.1046/j.1365-2427.2002.00931.x

Bláha, M., Grabicova, K., Shaliutina, O., Kubec, J., Randák, T., Zlabek, V., Buřič, M. & Veselý, L. (2019). Foraging behaviour of top predators mediated by pollution of psychoactive pharmaceuticals and effects on ecosystem stability. *The Science of the Total Environment*, 662, 655–661. https://doi.org/10.1016/j.scitotenv.2019.01.295

Cai, T., Park, S.Y. & Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews*, 19, 360–369. https://doi.org/10.1016/j.rser.2012.11.030

Chivers, D.P., Wisenden, B.D. & Smith, R.J.F. (1996). Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Animal Behaviour*, 52 (2), 315–320. https://doi.org/10.1006/anbe.1996.0177

Cooke, S.J., Midwood, J.D., Thiem, J.D., Klimley, P., Lucas, M.C., Thorstad, E.B., Eiler, J., Holbrook, C. & Ebner, B.C. (2013). Tracking animals in freshwater with electronic tags: past, present and future. *Animal Biotelemetry*, 1 (1), 5. https://doi.org/10.1186/2050-3385-1-5

Crump, A., Gibbons, M., Barrett, M., Birch, J. & Chittka, L. (2023). Is it time for insect researchers to consider their subjects' welfare? *PLOS Biology*, 21 (6), e3002138. https://doi.org/10.1371/journal.pbio.3002138

De Goede, D.M., Erens, J., Kapsomenou, E. & Peters, M. (2013). Large scale insect rearing and animal welfare. I: Röcklinsberg, H. & Sandin, P. (red.) *The ethics of consumption: The citizen, the market and the law*. Academic Publishers. 236–242. https://doi.org/10.3920/978-90-8686-784-4_38

Diesbourg, E.E., Kidd, K.A. & Perrotta, B.G. (2025). Effects of municipal wastewater effluents on the invertebrate microbiomes of an aquatic-riparian food web. *Environmental Pollution*, 372, 125948.

https://doi.org/10.1016/j.envpol.2025.125948
Donovan, T. & Iii, F. (2001). Modeling The Ecological Trap Hypothesis: A Habitat and Demographic Analysis for Migrant Songbirds. *Ecological Applications*, 11, 871–882. https://doi.org/10.2307/3061122
Escobar-Huerfano, F., Elizalde-Velázquez, G.A., Gómez-Oliván, L.M., Orozco-Hernández, J.M., Rosales-Pérez, K.E., Islas-Flores, H. & Hernández-Navarro, M.D. (2022). Environmentally relevant concentrations of fluconazole alter the embryonic development, oxidative status, and gene expression of NRF1, NRF2, WNT3A, WNT8A, NRD1, and NRD2 of Danio rerio embryos. *Water Emerging Contaminants & Nanoplastics*, 1 (1), N/A-N/A. https://doi.org/10.20517/wecn.2021.03
Gergs, A., Zenker, A., Grimm, V. & Preuss, T.G. (2013). Chemical and natural stressors combined: from cryptic effects to population extinction. *Scientific Reports*, 3 (1), 2036. https://doi.org/10.1038/srep02036
Gjerris, M., Gamborg, C. & Röcklinsberg, H. (2016). Ethical aspects of insect production for food and feed. https://doi.org/10.3920/JIFF2015.0097
Gogoi, A., Mazumder, P., Tyagi, V.K., Tushara Chaminda, G.G., An, A.K. & Kumar, M. (2018). Occurrence and fate of emerging contaminants in water environment: A review. *Groundwater for Sustainable Development*, 6, 169–180. https://doi.org/10.1016/j.gsd.2017.12.009
Gross, E.M. (2022). Aquatic chemical ecology meets ecotoxicology. *Aquatic Ecology*, 56 (2), 493–511. https://doi.org/10.1007/s10452-021-09938-2
Hadicke, C., Rédei, D. & Kment, P. (2017). The diversity of feeding habits recorded for water boatmen (Heteroptera: Corixoidea) world-wide with implications for evaluating information on the diet of aquatic insects. *European Journal of Entomology*, 114, 147–159. https://doi.org/10.14411/eje.2017.020
Hale, R. & Swearer, S. (2016). Ecological traps: Current evidence and future directions. *Proceedings of the Royal Society B: Biological Sciences*, 283, 20152647. https://doi.org/10.1098/rspb.2015.2647
Harrison, P.A., Vandewalle, M., Sykes, M.T., Berry, P.M., Bugter, R., de Bello, F., Feld, C.K., Grandin, U., Harrington, R., Haslett, J.R., Jongman, R.H.G., Luck, G.W., da Silva, P.M., Moora, M., Settele, J., Sousa, J.P. & Zobel, M. (2010). Identifying and prioritising services in European terrestrial and freshwater ecosystems. *Biodiversity and Conservation*, 19 (10), 2791–2821. https://doi.org/10.1007/s10531-010-9789-x
Havs- och Vattenmyndigheten (2014). *Kemiska bekämpningsmedel i grundvatten 1986–2014*. (2014:15). https://www.diva-portal.org/smash/get/diva2:1366726/FULLTEXT01.pdf
Heinloth, T., Uhlhorn, J. & Wernet, M.F. (2018). Insect Responses to Linearly Polarized Reflections: Orphan Behaviors Without Neural Circuits. *Frontiers in Cellular Neuroscience*, 12. https://doi.org/10.3389/fncel.2018.00050
Horváth, G., Kriska, G., Malik, P. & Robertson, B. (2009). Polarized light pollution: a new kind of ecological photopollution. *Frontiers in Ecology and the Environment*, 7 (6), 317–325. https://doi.org/10.1890/080129
van Huis, A. (2021). Welfare of farmed insects. https://doi.org/10.3920/JIFF2020.0061
Johnson, D.M. (1991). Behavioral ecology of larval dragonflies and damselflies. *Trends in Ecology & Evolution*, 6 (1), 8–13.

https://doi.org/10.1016/0169-5347(91)90140-S

Jonsson, M., Andersson, M., Fick, J., Brodin, T., Klaminder, J. & Piovano, S. (2019). High-speed imaging reveals how antihistamine exposure affects escape behaviours in aquatic insect prey. *Science of The Total Environment*, 648, 1257–1262. https://doi.org/10.1016/j.scitotenv.2018.08.226

Kraus, J.M. (2019). Contaminants in linked aquatic–terrestrial ecosystems: Predicting effects of aquatic pollution on adult aquatic insects and terrestrial insectivores. *Freshwater Science*, 38 (4), 919–927. https://doi.org/10.1086/705997

Let, M., Černý, J., Nováková, P., Ložek, F. & Bláha, M. (2022). Effects of Trace Metals and Municipal Wastewater on the Ephemeroptera, Plecoptera, and Trichoptera of a Stream Community. *Biology*, 11 (5), 648. https://doi.org/10.3390/biology11050648

McCallum, E.S., Krutzelmann, E., Brodin, T., Fick, J., Sundelin, A. & Balshine, S. (2017). Exposure to wastewater effluent affects fish behaviour and tissue-specific uptake of pharmaceuticals. *Science of The Total Environment*, 605–606, 578–588. https://doi.org/10.1016/j.scitotenv.2017.06.073

McCallum, E.S., Nikel, K.E., Mehdi, H., Du, S.N.N., Bowman, J.E., Midwood, J.D., Kidd, K.A., Scott, G.R. & Balshine, S. (2019). Municipal wastewater effluent affects fish communities: A multi-year study involving two wastewater treatment plants. *Environmental Pollution*, 252, 1730–1741. https://doi.org/10.1016/j.envpol.2019.06.075

McPeek, M.A. (1990). Behavioral Differences between Enallagma Species (Odonata) Influencing Differential Vulnerability to Predators. *Ecology*, 71 (5), 1714–1726. https://doi.org/10.2307/1937580

McPeek, M.A. & Crowley, P.H. (1987). The effects of density and relative size on the aggressive behaviour, movement and feeding of damselfly larvae (Odonata: Coenagrionidae). *Animal Behaviour*, 35 (4), 1051–1061. https://doi.org/10.1016/S0003-3472(87)80162-8

Mehdi, H., Lau, S.C., Synyshyn, C., Salena, M.G., McCallum, E.S., Muzzatti, M.N., Bowman, J.E., Mataya, K., Bragg, L.M., Servos, M.R., Kidd, K.A., Scott, G.R. & Balshine, S. (2021). Municipal wastewater as an ecological trap: Effects on fish communities across seasons. *Science of The Total Environment*, 759, 143430. https://doi.org/10.1016/j.scitotenv.2020.143430

Nassef, M., Matsumoto, S., Seki, M., Khalil, F., Kang, I.J., Shimasaki, Y., Oshima, Y. & Honjo, T. (2010). Acute effects of triclosan, diclofenac and carbamazepine on feeding performance of Japanese medaka fish (*Oryzias latipes*). *Chemosphere*, 80 (9), 1095–1100. https://doi.org/10.1016/j.chemosphere.2010.04.073

Naturskyddsföreningen (2021). *Sant eller falskt om ekologiskt*. https://www.naturskyddsforeningen.se/artiklar/sant-eller-falskt-om-ekologiskt/ [2025-03-11]

Nowińska, A., Franielczyk-Pietyra, B. & Polhemus, D.A. (2023). The Leg Sensilla of Insects from Different Habitats—Comparison of Strictly Aquatic and Riparian Bugs (Corixidae, Ochteridae, Gelastocoridae: Nepomorpha: Insecta: Heteroptera). *Insects*, 14 (5), 441. https://doi.org/10.3390/insects14050441

Previšić, A., Rožman, M., Mor, J.-R., Acuña, V., Serra-Compte, A., Petrović, M. & Sabater, S. (2020). Aquatic macroinvertebrates under stress:

Bioaccumulation of emerging contaminants and metabolomics implications. *Science of The Total Environment*, 704, 135333. https://doi.org/10.1016/j.scitotenv.2019.135333

Previšić, A., Vilenica, M., Vučković, N., Petrović, M. & Rožman, M. (2021). Aquatic Insects Transfer Pharmaceuticals and Endocrine Disruptors from Aquatic to Terrestrial Ecosystems. *Environmental Science & Technology*, 55 (6), 3736–3746. https://doi.org/10.1021/acs.est.0c07609

Rapp-Wright, H., Regan, F., White, B. & Barron, L.P. (2023). A year-long study of the occurrence and risk of over 140 contaminants of emerging concern in wastewater influent, effluent and receiving waters in the Republic of Ireland. *Science of The Total Environment*, 860, 160379. https://doi.org/10.1016/j.scitotenv.2022.160379

Rossi, S. (2024). Aquatic Invertebrates and Wastewater Effluents: Behavioral Responses and Ecological Implications Examining habitat preferences in aquatic invertebrates in northern Sweden.

Saaristo, M., Brodin, T., Balshine, S., Bertram, M.G., Brooks, B.W., Ehlman, S.M., McCallum, E.S., Sih, A., Sundin, J., Wong, B.B.M. & Arnold, K.E. (2018). Direct and indirect effects of chemical contaminants on the behaviour, ecology and evolution of wildlife. *Proceedings of the Royal Society B: Biological Sciences*, 285 (1885), 20181297. https://doi.org/10.1098/rspb.2018.1297

Salimi, M., Esrafili, A., Gholami, M., Jonidi Jafari, A., Rezaei Kalantary, R., Farzadkia, M., Kermani, M. & Sobhi, H.R. (2017). Contaminants of emerging concern: a review of new approach in AOP technologies. *Environmental Monitoring and Assessment*, 189 (8), 414. https://doi.org/10.1007/s10661-017-6097-x

Schmitz, O.J., Grabowski, J.H., Peckarsky, B.L., Preisser, E.L., Trussell, G.C. & Vonesh, J.R. (2008). From Individuals to Ecosystem Function: Toward an Integration of Evolutionary and Ecosystem Ecology. *Ecology*, 89 (9), 2436–2445. https://doi.org/10.1890/07-1030.1

Schowalter, T.D. (2022). *Insect Ecology: An Ecosystem Approach*. Academic Press.

Schuijt, L.M., van Drimmelen, C.K.E., Buijse, L.L., van Smeden, J., Wu, D., Boerwinkel, M.-C., Belgers, D.J.M., Matser, A.M., Roessink, I., Beentjes, K.K., Trimbos, K.B., Smidt, H. & Van den Brink, P.J. (2024). Assessing ecological responses to exposure to the antibiotic sulfamethoxazole in freshwater mesocosms. *Environmental Pollution*, 343, 123199. https://doi.org/10.1016/j.envpol.2023.123199

Scott, G.R. & Sloman, K.A. (2004). The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicology*, 68 (4), 369–392. https://doi.org/10.1016/j.aquatox.2004.03.016

Sibiya, A., Al-Ghanim, K.A., Govindarajan, M., Nicoletti, M., Sachivkina, N. & Vaseeharan, B. (2023). Biochemical Patterns and Genotoxicity of the Endocrine Disruptor Metformin in the Freshwater Fish Labeo rohita. *Fishes*, 8 (7), 380. https://doi.org/10.3390/fishes8070380

Smith, V.H., Joye, S.B. & Howarth, R.W. (2006). Eutrophication of Freshwater and Marine Ecosystems. *Limnology and Oceanography*, 51 (1), 351–355

Späth, J., Fick, J., McCallum, E., Cerveny, D., Nording, M.L. & Brodin, T. (2022). Wastewater effluent affects behaviour and metabolomic endpoints in damselfly larvae. *Scientific Reports*, 12 (1), 6830. https://doi.org/10.1038/s41598-022-10805-9

Stoks, R. (1999). Autotomy shapes the trade-off between seeking cover and foraging in larval damselflies. *Behavioral Ecology and Sociobiology*, 47 (1), 70–75. https://doi.org/10.1007/s002650050651

Van Praet, N., De Bruyn, L., De Jonge, M., Vanhaecke, L., Stoks, R. & Bervoets, L. (2014). Can damselfly larvae (*Ischnura elegans*) be used as bioindicators of sublethal effects of environmental contamination? *Aquatic Toxicology*, 154, 270–277. https://doi.org/10.1016/j.aquatox.2014.05.028

Villalobos-Jimenez, G., Dunn, A.M. & Hassall, C. (2016). Dragonflies and damselflies (Odonata) in urban ecosystems: A review. *European Journal of Entomology*, 113, 217–232. https://doi.org/10.14411/eje.2016.027

Vonesh, J. & Kraus, J. (2009). Pesticide alters habitat selection and aquatic community composition. *Oecologia*, 160, 379–85. https://doi.org/10.1007/s00442-009-1301-5

Wallin, S., Halldin Ankarberg, E. & Fohgelberg, P. (2012). *Resthalter och tidstrend av bekämpningsmedlet endosulfan i livsmedel samt uppskattat intag för den svenska konsumenten*. (Dnr 235-1781-08Mm). https://www.diva-portal.org/smash/get/diva2:711361/FULLTEXT01.pdf

Ward, A.J.W., Duff, A.J., Horsfall, J.S. & Currie, S. (2007). Scents and scents-ability: pollution disrupts chemical social recognition and shoaling in fish. *Proceedings of the Royal Society B: Biological Sciences*, 275 (1630), 101–105. https://doi.org/10.1098/rspb.2007.1283

Wiles, S.C., Bertram, M.G., Martin, J.M., Tan, H., Lehtonen, T.K. & Wong, B.B.M. (2020). Long-Term Pharmaceutical Contamination and Temperature Stress Disrupt Fish Behavior. *Environmental Science & Technology*, 54 (13), 8072–8082. https://doi.org/10.1021/acs.est.0c01625

Wurtsbaugh, W.A., Paerl, H.W. & Dodds, W.K. (2019). Nutrients, eutrophication and harmful algal blooms along the freshwater to marine continuum. *WIREs Water*, 6 (5), e1373. https://doi.org/10.1002/wat2.1373

Xie, Z., Gan, Y., Tang, J., Fan, S., Wu, X., Li, X., Cheng, H. & Tang, J. (2020). Combined effects of environmentally relevant concentrations of diclofenac and cadmium on Chironomus riparius larvae. *Ecotoxicology and Environmental Safety*, 202, 110906. https://doi.org/10.1016/j.ecoenv.2020.110906

Appendix 1

Water sample analysis results, previously published by Rossi (2024). Two samples from the wasterwater used in the experiments (Wastewater 1 and Wastewater 2) and a sample of the tap water used in the holding tanks, as well as in the experiments, were analysed regarding their chemical compouds.

	LabID	24	25	5	6
	SampleID	BLANK	Tap water	Wastewater 1	Wastewater 2
	Project	MillQ water	Tap water	Sarah Rossi Thesis	Sarah Rossi Thesis
	SampleDate	NA	23.11.2023	16.06.2023	16.06.2023
	Sample mL	150.04	150.02	150	150
	Extraction date	November 13 2023	November 13 2023	November 10 2023	November 10 2023
concentration ng/L					
concentration ng/L	**Alfuzosin**	<LOQ	<LOQ	50.43	50.93
concentration ng/L	**Alprazolam**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Amitryptiline**	<LOQ	<LOQ	55.37	71.16
concentration ng/L	**Atenolol**	<LOQ	<LOQ	897.30	1055.67
concentration ng/L	**Atorvastatin**	<LOQ	<LOQ	264.48	361.36
concentration ng/L	**Atracurium**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Azithromycine**	<LOQ	<LOQ	40.294	32.51
concentration ng/L	**Beclomethazone**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Bisoprolol**	<LOQ	<LOQ	96.35	99.16
concentration ng/L	**Budesonide**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Buprenorphin**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Bupropion**	<LOQ	<LOQ	36.84	34.30
concentration ng/L	**Caffeine**	11.12	10.80	439.14	466.07
concentration ng/L	**Carbamazepin**	<LOQ	<LOQ	254.40	282.12
concentration ng/L	**Citalopram**	<LOQ	<LOQ	90.95	93.33
concentration ng/L	**Clarithromycine**	<LOQ	<LOQ	33.60	43.65
concentration ng/L	**Clindamycine**	<LOQ	<LOQ	101.82	108.93
concentration ng/L	**Clonazepam**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Codeine**	<LOQ	<LOQ	103.62	91.25
concentration ng/L	**Desloratidin**	<LOQ	<LOQ	68.27	68.65
concentration ng/L	**Diclofenac**	<LOQ	<LOQ	435.32	478.78
concentration ng/L	**Dicycloverin**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Dihydroergotamin**	<LOQ	<LOQ	<LOQ	<LOQ

concentration ng/L	**Diltiazem**	<LOQ	<LOQ	6.45	7.74
concentration ng/L	**Diphenhydramin**	<LOQ	<LOQ	5.92	6.64
concentration ng/L	**Dipyridamol**	<LOQ	<LOQ	14.02	24.84
concentration ng/L	**Donepezil**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Eprosartan**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Erythromycine**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Etonorgestrel**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Ezetimibe**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Felodipine**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Fenofibrate**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Finasteride**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Flecainide**	<LOQ	<LOQ	51.08	57.60
concentration ng/L	**Fluconazole**	<LOQ	<LOQ	180.27	168.36
concentration ng/L	**flunitrazepam**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Fluoxetin**	<LOQ	<LOQ	11.14	5.05
concentration ng/L	**Flupentixol**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Fluphenazine**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Flutamid**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Glibenclamide**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Glimepiride**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Haloperidol**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Ibersartan**	<LOQ	<LOQ	91.16	125.62
concentration ng/L	**Ketoconazole**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Memantin**	<LOQ	<LOQ	44.77	44.50
concentration ng/L	**Metformin**	<LOQ	<LOQ	2568.58	2378.79
concentration ng/L	**Metoprolol**	<LOQ	<LOQ	1619.41	1687.27
concentration ng/L	**Mianserin**	<LOQ	<LOQ	4.89	8.70
concentration ng/L	**Miconazole**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Mirtazapine**	<LOQ	<LOQ	156.59	184.59
concentration ng/L	**Naloxon**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Orphenadrin**	<LOQ	<LOQ	12.52	16.94
concentration ng/L	**Oxazepam**	<LOQ	<LOQ	129.15	134.76
concentration ng/L	**paracetamol**	<LOQ	<LOQ	24.06	24.78
concentration ng/L	**Perphenazine**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Propranolol**	<LOQ	<LOQ	75.81	67.46
concentration ng/L	**Ranitidine**	<LOQ	<LOQ	<LOQ	<LOQ

concentration ng/L	**Repaglinide**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Risperidone**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Rosuvastatin**	<LOQ	<LOQ	2623.31	2635.25
concentration ng/L	**Roxithromycine**	<LOQ	<LOQ	<LOQ	<LOQ
concentration ng/L	**Sertraline**	<LOQ	<LOQ	73.25	109.51
concentration ng/L	**Sulfamethoxazol**	<LOQ	<LOQ	637.91	810.65
concentration ng/L	**Tetracycline**	<LOQ	<LOQ	141.20	163.95
concentration ng/L	**Tramadol**	<LOQ	<LOQ	864.85	892.05
concentration ng/L	**Trimetoprim**	<LOQ	<LOQ	408.35	412.17
concentration ng/L	**Venlavafaxin**	<LOQ	<LOQ	192.31	202.30
concentration ng/L	**Verapamil**	<LOQ	<LOQ	6.59	6.90
concentration ng/L	**Zolpidem**	<LOQ	<LOQ	1.70	1.20

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