



# Residence of four wild fish species near a wastewater treatment plant outfall in southern Sweden

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Degree project • 30 credits

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Conservation and Management of Fish and Wildlife MSc

Umeå, Sweden 2024



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**Credits:** 30  
**Level:** Master's level (A2E)  
**Course title:** Master's thesis in Biology, A2E - Wildlife, Fish, and Environmental Studies  
**Course code:** EX0971  
**Programme/education:** Conservation and Management of Fish and Wildlife MSc  
**Course coordinating dept:** Department of Wildlife, Fish and Environmental Studies  
**Place of publication:** Umeå, Sweden  
**Year of publication:** 2024  
**Copyright:** All featured images are used with permission from the copyright owner.  
**Keywords:** wastewater, wastewater treatment plant, weighted residency index, acoustic telemetry, fish, Sweden

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## Abstract

Wastewater discharge from wastewater treatment plants (WWTP) is known to have physiological and behavioral impacts on fish. Despite this, there is limited knowledge of fish residency patterns near WWTP outfalls in the wild. We used acoustic telemetry to examine the residency patterns of common bream (*Abramis brama*), northern pike (*Esox lucius*), zander (*Sander lucioperca*), common rudd (*Scardinius erythrophthalmus*), and their proximity to a WWTP outfall in southern Sweden between spring and fall. We found that pike exhibited a significant preference for the outfall. Rudd showed a significant preference for the upstream site over the downstream site across all months between May and October and a significant preference for the upstream site over all other sites in May, June, July, and September. Site-specific differences in the mean water temperature range were found only in June, suggesting that in May, July, August, and September, the mean water temperature range was unlikely to have a primary influence on fish habitat choice.

Keywords: wastewater, wastewater treatment plant, weighted residency index, acoustic telemetry, fish, Sweden

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# 1. Introduction

European riverine habitats have been altered by extended exposure to anthropogenic activity (Nilsson et al., 2005). The release of synthetic chemicals into ecosystems is increasing due to an increasing human population and its growing use of chemicals (Bernhardt et al., 2017; Schwarzenbach et al. 2006). Wastewater treatment plants (WWTPs) are a contributor to aquatic pollution (Horii et al., 2007; Loganathan et al., 2007; Nakada et al., 2004; Schwarzenbach et al., 2010; Stevens et al., 2003), releasing contaminants of emerging concern (CECs) into aquatic ecosystems (Barbosa et al. 2016; Chinnaiyan et al. 2018; Liu et al. 2018). Various contaminants are found in WWTP effluent, including pharmaceuticals and personal care products (PPCPs), hormones, excess nutrients, and pesticides (He et al., 2023; Holeyton et al., 2011; Jucyte-Cicine et al., 2024; Loos et al., 2013; McCormick et al., 2016; Rodriguez-Mozaz et al., 2020). While WWTPs remove portions of some contaminants from their effluent during conventional treatment processes, they do not eliminate them (Gargosova & Urminska, 2017; Batt & Aga, 2005; Carballa et al., 2004; Costanzo et al., 2005). WWTP effluent can also alter various aspects of the receiving environment, such as salinity (Noyes et al., 2009), bacteria levels (Peterson et al., 2005), water temperature (Hamdhani et al., 2020), and the organic carbon content (McConnell, 1980). Moreover, excess nutrients from WWTP effluent can contribute to eutrophication (Carey & Migliaccio, 2009) and disrupt ecosystem functioning in aquatic ecosystems (Rabalais, 2002).

High concentrations of certain pollutants have been identified at outfalls (McCallum et al., 2017a; Moon et al., 2008; Wiest et al., 2021) and, when exposed to wastewater effluent, fish can uptake waterborne pollutants into their tissues, leading to impacts on their physiology and behavior (Grabicova et al., 2014; McCallum et al., 2017b, 2019; Tanoue et al., 2015). Fish exposure to wastewater pollutants has been linked to increased resting metabolic rate (Mehdi et al., 2018), reduced liver and muscle glycogen (Cazenave et al., 2014), reduced whole-body lipids (Melvin et al., 2016), and an altered liver somatic index (Yeom et al., 2007). Exposure to wastewater effluents or wastewater contaminants has also been reported to influence fish behavior (Scott & Sloman, 2004), including reduced swimming capability (Little & Finger, 1990; Melvin, 2016), lowered competitiveness ability (Martinovic et al., 2007), reduced foraging behavior (Kasumyan, 2001), increased boldness and activity (Brodin et al., 2013, 2017), and increased downstream migration rates (McCallum et al., 2019). As some behavioral changes in fish, such as reduced or delayed predator recognition and avoidance (McLean et al., 2019; Pelli & Connaughton, 2015), can affect survival (Dugatkin, 1992), these behavioral changes could impact fish at the population and community level (Weis et al., 2001).

Despite the widely reported negative impacts of WWTP contaminants on fish, studies have found higher fish abundance and species richness near outfalls (Azzurro et al., 2010; Grigg, 1994; McCallum et al., 2019b; Mehdi et al., 2021). Previous work has suggested that fish might be attracted to outfalls (Azzurro et al., 2010; McCallum et al., 2019; Mehdi et al., 2021) due to the favorable conditions created by wastewater effluent, which creates a thermally stable environment (Cooke et al., 2004; Dryer & Benson, 1957; Kinouchi et al., 2007; Mehdi et al., 2019, 2021) and contributes organic matter that can attract prey (Guidetti et al., 2003; Grigg, 1994; Gray 1996, 1997; Hall et al., 1997; Northington & Hershey, 2006). Increases in and stabilization of receiving water temperatures due to WWTP effluent have been specifically noted at WWTP outfalls (Kinouchi et al., 2007; Mehdi et al., 2021), and wild fish have been observed selecting areas near outfalls where the effluent was heated and temperature-regulated (Cooke et al., 2004; Dryer & Benson, 1957; Mehdi et al., 2021). Another appealing aspect of outfalls may be that they act as visual landmarks for fish, helping fish navigate towards resource patches (Wolfe & Lowe, 2015). However, although wastewater outfalls may be attractive to fish, not all fish may tolerate the altered water quality around outfalls, potentially leading to outfalls favoring individuals with certain characteristics. In this way, fish communities and assemblage structures downstream from wastewater outfalls can be altered by wastewater inputs (Porter & Janz 2003; Ra et al. 2007; Yoem et al. 2007). Specifically, fish communities near WWTP outfalls have been identified to include more pollution-tolerant, stress-tolerant, omnivorous, and non-native species (Dyer & Wang, 2002; Kornis et al., 2012; McCallum et al., 2019; Ra et al., 2007; Reash & Berra, 1987; Stephens et al., 1988; Tetreault et al., 2013).

Water temperature changes near WWTP outfalls may have consequences for resident fish populations. Water temperature is a critical variable influencing the sustainability of fish populations (Kappenman et al., 2009; Phelps et al., 2010), affecting their survival (Kappenman et al., 2009; Payne et al., 2016), physiology (Hochachka & Somero 2002; Luksiene & Svedang, 1997; Pörtner & Knust, 2007), and behavior (Zhang et al., 2020). Elevated water temperatures have been linked to varying changes in fish activity. Some studies have linked decreased activity with temperature increases (Johansen et al., 2014; Scott et al., 2017). In contrast, other studies observed increased activity with increased temperature (Klinard et al., 2018; Topping et al., 2006), although Nolan et al. (2024) report that beyond 15 °C, the positive correlation between temperature and activity ceases for northern pike and zander, and further temperature increases beyond this threshold result in decreased activity. This suggests that the impact of temperature on fish behavior may vary depending on the species and the extent of the temperature increase. Furthermore, water temperature can influence wild fish presence and absence (Cooke & McKinley, 1999; Kessel et al., 2016; Stebbing et al., 2002). For instance, a study at a steam-electric power plant outfall indicated

that water temperature was the primary factor affecting fish distribution. Fish were more concentrated in the outfall area during summers when the water temperatures at the outfall were consistent with those of other summers (Neill & Magnuson, 1974). Additionally, increased water temperatures downstream of dam release sites have also been associated with lower densities of cold-water fish species, further illustrating the impact of thermal changes on fish at a community level (Lessard et al., 2003).

There is limited knowledge of how WWTP outfalls influence fish habitat choice and residency, particularly in Sweden. While previous research has examined the effects of WWTP contaminants on fish in laboratory settings and tracked fish movements near Swedish hydropower plants (Calles et al., 2012; Hellström et al., 2016; Klaminder et al., 2019; McCallum et al., 2019; Nyqvist et al., 2018), a knowledge gap remains regarding the specific impacts of WWTP outfalls on fish in natural settings. To address this knowledge gap, fish movements should be tracked in situ. Acoustic telemetry is an effective method for achieving this at ecologically relevant scales. This technique is widely used in the study of fish movement ecology because it enables researchers to monitor aquatic species autonomously in their natural environments (Heupel et al., 2006; Matley et al., 2022; Vianna et al., 2014; Williamson et al., 2020). In this method, an electronic transmitter is attached or implanted into individuals. These transmitters emit high-frequency acoustic signals that can be detected when the tagged fish are within the range of an acoustic receiver (Hoenner et al., 2018; Heupel et al., 2006). Each detection record contains the transmitter's unique code, along with the time, date, and additional data specific to each receiver, such as the water temperature or the angle of the receiver (Innovasea Systems Inc., 2020). Acoustic telemetry is particularly suitable for studying the relationships between fish residency, water temperature, and proximity to outfalls, as it allows for continuous, long-term monitoring in situ (Donaldson et al., 2014; Heupel & Webber, 2012; Hussey et al., 2015; Matley et al., 2024; Kraft et al., 2023). An advantage of this method is the large amounts of data that can be generated and minimal maintenance needs after the initial setup (Heupel et al., 2006). Moreover, acoustic telemetry data can be integrated with other environmental datasets, such as streamflow data, pH, or dissolved oxygen data, allowing for comprehensive studies of ecological dynamics.

This study used acoustic telemetry to examine the weighted residency index (RI) of four fish species with a WWTP outfall and water temperature range values in a Swedish river. RI represents the proportion of time an individual is present within an area during a designated period with values ranging from 0, which indicates no residency, to 1, indicating continuous presence throughout the study period (Afonso et al., 2008). We used the weighted RI, which calculates a more accurate representation of residency than the standard RI because it weights the index by the period between the first and last detections (Lino, 2012).

This study aimed to answer the following questions:

- 1) Does the monthly mean water temperature range vary upstream, downstream, and at a WWTP outfall in a southern Swedish river system?
- 2) Do fish residency patterns differ between species and between the outfall, upstream, and downstream sites?

Given that WWTP effluent can have thermal regulation effects (Kinouchi et al., 2007; Mehdi et al., 2021), I expected that the monthly mean water temperature range in various sites would be affected by proximity to the WWTP outfall. Therefore, I predicted that throughout the study period, the monthly mean water temperature range would be greatest upstream from the outfall, where the thermal regulation of wastewater effluent is less pronounced. Since WWTP effluents can create thermal refuges (Dryer & Benson, 1957; Kinouchi et al., 2007) and increase nutrient availability (Grigg, 1994; Guidetti et al., 2003; Mehdi et al., 2021), I anticipate that fish will be attracted to the outfall for the conditions created by the wastewater discharge. Therefore, I predict that the weekly weighted RI of fish will be highest at the outfall site, regardless of species.

## 2. Methodology

### 2.1 Study area

The study area was located in the lower portion of Arboga River at the outfall of Arboga Vatten och Avlopp WWTP (heretofore referred to as Arboga WWTP) in Västmanland County, Sweden (59.399° N, 15.8831° E, WGS84, Fig. 1). Arboga River is 45 km long, excluding source flows, and one of the largest tributaries of Lake Mälaren (Mälarens Vattenvårdsförbund, 2024). Further upstream from the study area is the Grind Berga power plant, which releases controlled amounts of water intermittently into Arboga River (Pettersen, 2009).



Figure 1. The study area is located in southern Sweden. The map is rendered in EPSG:3857 - WGS 84 / Pseudo-Mercator coordinate system

## 2.2 Deploying the acoustic receiver array

At the confluence with the outfall of Arboga WWTP, thirteen VEMCO HR2 receivers were deployed (receiver frequency 180 kHz, 40 cm length, 10 cm diameter, 2.88kg in the air with lithium battery, six-month battery life with lithium battery, data capacity 170,000,000 detections, operating temperature -5C - 40C, maximum depth 300 m, Innovasea Systems Inc., 2018, Fig. 2). Each receiver was outfitted with a circular donut buoy and tied to a weight with 0.5 - 1 m rope. This ensured the receiver stayed anchored in place underwater, remained floating vertically in the water column, and was positioned away from the sediment. From April 29, 2023 to October 6, 2023 the receivers were deployed along a 3.22 km length at the Arboga WWTP outfall, as well as upstream and downstream from the outfall. The design of the receiver array ensured that the upstream, downstream, and outfall sites did not have overlapping detection ranges. The array was designed to have 100 % detection coverage with the aim of detecting all tagged individuals passing through the outfall area (Barry et al., 2020; Heupel et al., 2006; How & de Lestang, 2012; Le Pichon et al., 2017; Welch et al. 2008). The high number of receivers at the outfall also enabled the detection of fish movements at finer, high-resolution spatial scales via triangulation; however, this analysis is not part of the data analyzed and presented in this thesis (Cooke et al., 2005; Espinoza et al., 2011). The receivers upstream and downstream were positioned to act as gates that detected fish entering and exiting the array. All receivers were programmed to operate in continuous receiving mode and were set to record the tilt of the receiver (°) and the water temperature (°C) every ten minutes.

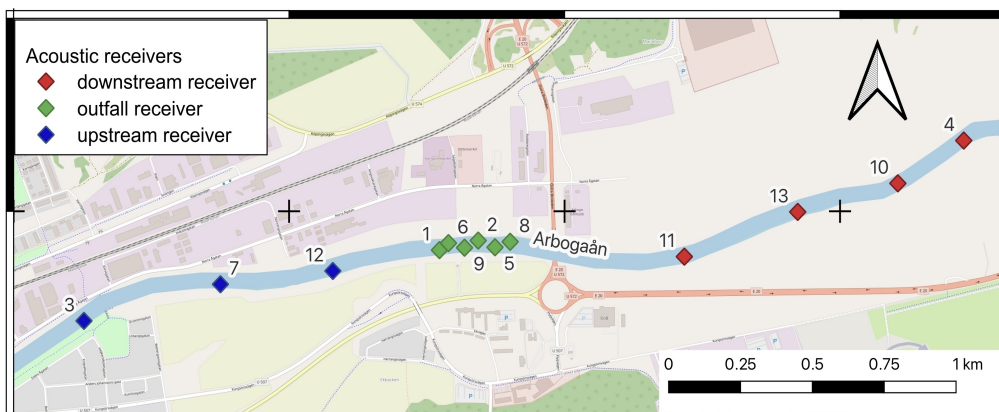


Figure 2. The distribution of the Vemco V9 acoustic receivers deployed at the study site located at Arboga River in Västmanland County, Sweden at 59.399° N, 15.8831° E (WGS84). The map is rendered in EPSG:3857 - WGS 84 / Pseudo-Mercator coordinate system.

To assess the performance and detection range of the array, 5 Vemco V9-180 kHz coded transmitters were deployed as reference tags. These reference tags were anchored to draglines tied to five of the six outfall receivers. The reference tags were programmed to transmit a signal every three seconds, matching the

transmission interval of the transmitters implanted in the tagged fish (Melnychuk, 2012). The short pulse train of 3-5 seconds used by the reference transmitters ensured minimal interference with transmissions from the tagged fish (Melnychuk, 2012). These reference tags allowed us to assess the functionality of the array in capturing and recording transmissions even when tagged fish were not present in the array by calculating the detection efficiency of each outfall receiver at various distances (Kessel et al., 2016; Melnychuk, 2012). By comparing the number of recorded detections to the number of known signals sent by the reference tags, the detection efficiency of each outfall receiver was assessed (Starr et al., 2000).

## 2.3 Fish collection and tagging

On April 29th, 2023 and April 30th, 2023, 80 fish were electrofished from a boat by an electrofishing contractor. They were collected at the Arboga WWTP outfall (n = 40) and upstream from the outfall (n = 40). The fish were collected under a permit from the Västmanland County Administrative Board, which allowed us to conduct this fieldwork. Following their capture, the fish were temporarily transferred to an aerated tank at the river's edge, filled with river water and maintained with a battery-powered aerator.

Out of the 80 transmitters, one was found to be non-operational, resulting in 79 tagged individuals. The tagged species included common bream (*Abramis brama*, n = 29), northern pike (*Esox lucius*, n = 31), zander (*Sander lucioperca*, n = 9), common rudd (*Scardinius erythrophthalmus*, n = 8), and roach (*Rutilus rutilus*, n = 2). Of these tagged individuals, 40 were collected from the outfall and 39 were collected from the upstream site. The fish were tagged using Vemco V9-180 kHz coded transmitters (transmitter family V9-180 kHz, 26mm length, 9mm diameter, 3.7g in air, 2.9g in water, power output 143dB, Innovasea Systems Inc., 2020). These transmitters were coded to transmit their unique ID codes every three seconds using High Residence (HR) and Pulse Position Modulation (PPM) transmission systems (Innovasea Systems Inc., 2020). The three-second frequency was selected to maximize the battery life of the transmitters, ensuring they would last throughout the study period while still providing high-resolution data.

Each fish was anesthetized by immersion in a water solution of tricaine methanesulfonate (MS-222) and was monitored until its breathing slowed and it became unresponsive to touch. The weight (g), species, and total length (TL, in cm) of each fish were recorded. We also documented the serial number and ID of each transmitter, the tagging date, and the collection site. Before implantation, all tags, tools, and suture threads were sterilized by submergence in 95% ethanol. A Vemco V9-180 acoustic transmitter was then implanted into the intraperitoneal cavity through a small incision, which was subsequently closed with two sutures

of sterile surgical thread (size 2.0 or 3.0, depending on the species). Fish with TL ranging from 21 cm to 50 cm were tagged and the tag burdens of the transmitters varied from 0.4% to 3.9% of fish body weights. After surgery, the fish were placed in a dark, aerated recovery bin filled with river water from the collection site, where they were monitored during recovery from anesthesia. Fish were considered recovered when they regained buoyancy and responded to an approaching hand. All tagging procedures followed Jordbruksverket's standards (ethical permit # A 5-2023).

Upon recovery, the fish were held overnight in a keep net in the river before being released the following day. Keeping the fish overnight before releasing them may have helped contain the unusual behaviors and physiological changes that fish can exhibit up to 24 hours after being electrofished (Burns & Lantz, 1978; Mesa & Schreck, 1989). Placing the keep net in the river and in temperatures close to the source water temperature was also intended to support their physiological recovery (Aslanidi et al., 2008; Donaldson et al., 2008; Suski et al., 2006; Shultz et al., 2011). Likewise, handling and releasing the fish near their capture and tagging sites also minimized handling time outside the water (Brownscombe et al., 2019). On April 30, 2023 and May 1, 2023, the tagged fish were released at the same sites from which they were originally collected.

## 2.4 Data analysis

Data analyses and plots were conducted using the statistical software R (Version 4.2.3; R Core Team, 2023). To avoid using detections of tagged fish while they were kept in keepnets overnight on April 29 and 30, 2023, these dates were removed from the dataset, which resulted in the removal of three fish (IDs 64103, 64211, 64207) that left the array immediately after release and within those two dates. During the 158-day study period from May 1, 2023 to October 5, 2023, the array recorded 4,483,876 detections of tagged fish.

Vemco receivers have false detections from two main sources: from transmitters of tagged individuals and the collision of transmissions from multiple sources (Simpfendorfer et al., 2015), creating ID codes that do not match any from the study (Heupel et al., 2006; Kraft et al., 2023; Voegeli et al., 2001). By filtering only for known IDs of tagged individuals, the second type of false detection was eliminated from the dataset. To eliminate the first type of false detection, a fish was assumed to be present at a receiver only if at least two detections were recorded by that receiver (Gutowsky et al. 2013; Kneebone et al., 2012; Lee et al. 2015; Wolfe & Lowe, 2015). Therefore, I used the function `findSolo()` from package `remotes` in R Studio to eliminate single-hit false detections that were isolated sequentially on either side by a one-hour period at a receiver (Clements et al., 2005; Kessel et al., 2016). As such, 3,623 false detections were discarded from

the detection dataset, which accounted for 0.08% of the total detections.

Further data cleaning involved examining abacus plots for cessation of movement, as cessation of movement in tagged fish often indicates mortality, predation, or tag loss (Cooke et al., 2011; Hightower et al., 2001; Jepsen et al., 2002; Karam et al., 2008; Lacroix et al., 2004; Nowell et al., 2015; Sammons & Glover, 2013; Waters et al., 2005). Consequently, two individuals were presumed dead and removed (IDs 64269 and 64264). Afterwards, the single remaining roach was excluded from the analysis, as its small sample size would not provide sufficient data for analysis. As fish with ID 64273 was never detected, the final analysis included 4,369,593 detections from 72 individuals: common bream (n = 26), northern pike (n = 29), zander (n = 9), and roach (n = 8).

To examine the differences in the water temperature range for each site, water temperature data were obtained from readings recorded every ten minutes by the receivers. We calculated the daily water temperature ranges for each combination of receiver and day by subtracting the minimum water temperatures from the maximum water temperatures. To analyze the differences in water temperature range across the different sites, we first fit a model assessing the effect of site and day on the mean water temperature range values. The analysis revealed issues with clear periodicity and temporal autocorrelation of the response variable. To reduce the influence of temporal autocorrelation, we broke the analysis into five models, one for each month. We used generalized linear mixed models (GLMMs) using the `glmmTMB` function from the `glmmTMB` package and the `lmer` function from the `lme4` package. We then used the `simulateResiduals` function and the `plotResiduals` function from the `DHARMA` package to assess model fit. We used the `drop1` function from the `MASS` package to run a likelihood ratio test to assess whether the inclusion of interaction terms significantly improved model fit. Then, the assumptions of these models were also tested using the Shapiro-Wilk test, Quantile-Quantile (Q-Q) plots, and the Breusch-Pagan test. Lastly, estimated marginal means (EMMs) post hoc tests from the `emmeans` package were used to compare mean water temperature range differences between pairs of sites for each month. The model with the best fit for May, June, and September used the water temperature range as the response variable site and day as categorical predictor variables. For July and August, the best-fitting model also used the water temperature range as the response variable site and day as categorical predictor variables, but additionally performed a logarithmic transformation on the water temperature range values to meet linear model assumptions. Statistical significance was accepted at  $p < 0.05$ .

To measure fish residency, we used RI, a widely used metric for evaluating the number of days an individual was present within an array (Afonso et al., 2008; Appert et al., 2023; Novak et al., 2020). While there are several methods for calculating RI, we chose to use weighted RI for this study because it addresses the assumptions and mitigates the biases and overestimations of other

RI calculations (Cochran et al., 2019; Kraft et al., 2023; Lino, 2012). Weighted RI accounts for tag loss by requiring a final detection of a transmitter to complete the monitoring period, which weights the standard RI value with the duration during which it is known that a tagged individual was alive and the tag was operational (Cochran et al., 2019). In this way, weighted RI ensures that the RI value is not biased by periods of few but consecutive detections or by periods of no detections (Kraft et al., 2023). To investigate whether fish residency patterns varied by site and species, a weighted RI dataset was created using the following equation from Kraft et al. (2023):

$$\text{Weighted RI} = (\text{Dd}/\text{Dt}) \times (\text{Di}/\text{Dt})$$

This weighted RI calculation uses two fractions and multiplies them to find the weighted RI. The first fraction includes the **total number of days an individual is detected as present (Dd)** divided by the **total number of monitoring days in the study period (Dt)**. The second fraction includes the **period between the first and last detections of an individual (Di)** divided by the **total number of monitoring days in the study period (Dt)** (Kraft et al., 2023). Fish absent during a given week at a given site were given a weekly weighted RI of 0. To minimize the effects of false positive detections, a tagged fish was considered resident at a site for a given day if it was detected at least twice consecutively on any receiver at that site during that day (Kneebone et al., 2012; Lédée et al., 2015; Novak et al., 2020; Wolfe & Lowe, 2015). Using this daily presence-absence data, we calculated the weekly weighted RI values for each tagged individual at each site (outfall, upstream, and downstream) for each week of the study period.

To assess the effects of species, site, and month on the weekly weighted RI of fish, we used a GLMM with a beta distribution, which is appropriate for continuous data bounded between 0 and 1. Then, to simplify the interpretation of the three-way interaction, we divided the dataset into months before applying a second GLMM to each monthly dataset. (Note: Zander were never detected in the array after June, and were therefore removed from comparisons after this month). This second GLMM included site, species, and their interactions as fixed effects. Fish ID was incorporated as a random effect to account for each fish being represented multiple times in the analysis, and the model assumed an ordinal beta distribution with a logit link function. Post hoc analysis involved using EMMs to compare weighted RI values between sites within each species and between species at each site. We assessed model fit following the same steps as described above for the water temperature range analysis. Statistical significance was accepted at  $p < 0.05$ .

## 3. Results

### 3.1 Descriptive statistics

Zander had the highest mean TL ( $44.60\text{cm} \pm 2.16\text{cm}$ ) and highest mean body weight ( $761.56\text{g} \pm 110.03\text{g}$ ) among all 4 species while rudd had the lowest mean body weight ( $300.50\text{g} \pm 148.08\text{g}$ ) and lowest mean TL ( $26.90 \pm 4.01$ ) (Table 1).

Table 1. The total length (TL, in cm) and body weight (g) of each species of fish used in the final analysis.

Species	N	Mean body weight	SD weight	Mean TL	SD TL
Bream	26	375.27	206.42	31.1	4.66
Pike	29	398.52	150.08	39.4	5.52
Rudd	9	300.5	148.08	26.9	4.01
Zander	8	761.56	110.03	44.6	2.16

Rudd spent the highest total number of days in the array, on average ( $119.50 \pm 51.10$ ), while zander were present for the fewest total number of days, on average ( $19.11 \pm 10.24$ ). Pike were present for an average of  $81.68 \pm 65.70$  days, and bream were present for an average of  $51.54 \pm 74.34$  days. Rudd also spent the highest median number of days in the array among all species (Fig. 3).

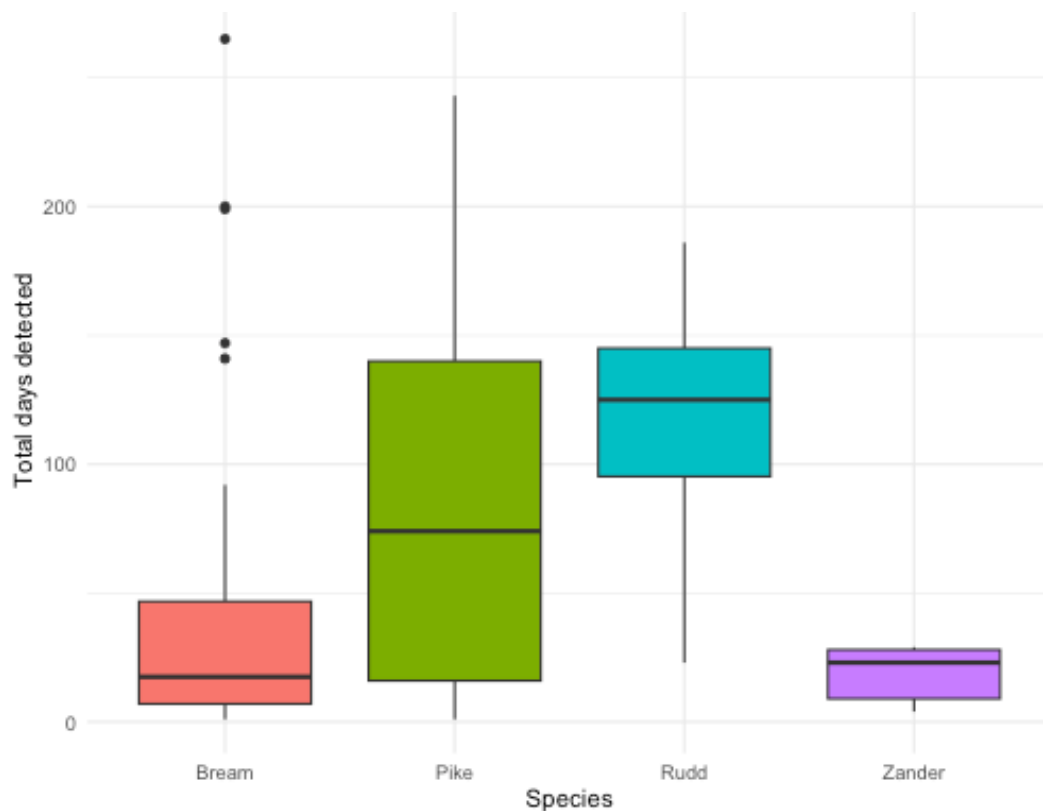


Figure 3. Boxplots show the interquartile range of the number of days detected in the array per species of fish included in the final analysis. The median lines indicate the median number of days detected in the array per species. The top edge of each box indicates the 75th percentile and the bottom edge indicates the 25th percentile. The whiskers extend to the data points that are within 1.5 times the interquartile range. Data points outside of this range are marked as individual dots.

The mean daily detections per fish and month of the study, were highest in September ( $45,062.50 \pm 10,321.05$ ), followed by May ( $29,807.74 \pm 5,524.53$ ), August ( $25,589.81 \pm 6,577.98$ ), July ( $19,515.97 \pm 5,393.99$ ), then June ( $17,541.27 \pm 2,274.92$ ). The median daily detections per fish per month are shown below (Fig. 4).

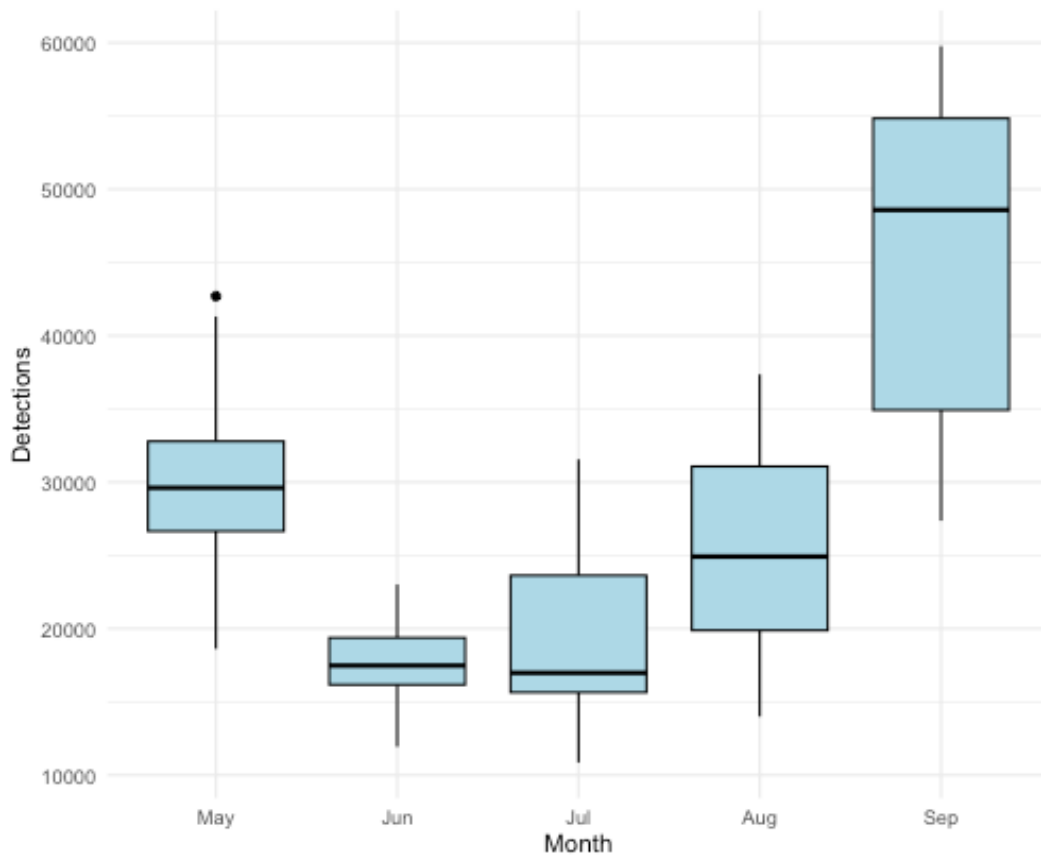


Figure 4. The distribution of the total number of detections per day across different months (summed across all individuals and receivers). For each month, the plot uses the total number of detections calculated for each day and shows how daily detection counts vary throughout the month. The boxplots show the interquartile range of the total number of detections per month of only the fish included in the final analysis. The median lines indicate the median number of detections of tagged fish each month. The top edge of each box indicates the 75th percentile and the bottom edge indicates the 25th percentile. The whiskers extend to the data points that are within 1.5 times the interquartile range.

50% of tagged fish ( $n = 36$ ) were detected in the array for 28 or fewer days. 25% of the fish ( $n = 18$ ) were detected for 9 days or fewer. The fish with the highest number of days detected in the array for any individual was detected for 153 days out of the total 158 days that we ran the study (Fig. 5).

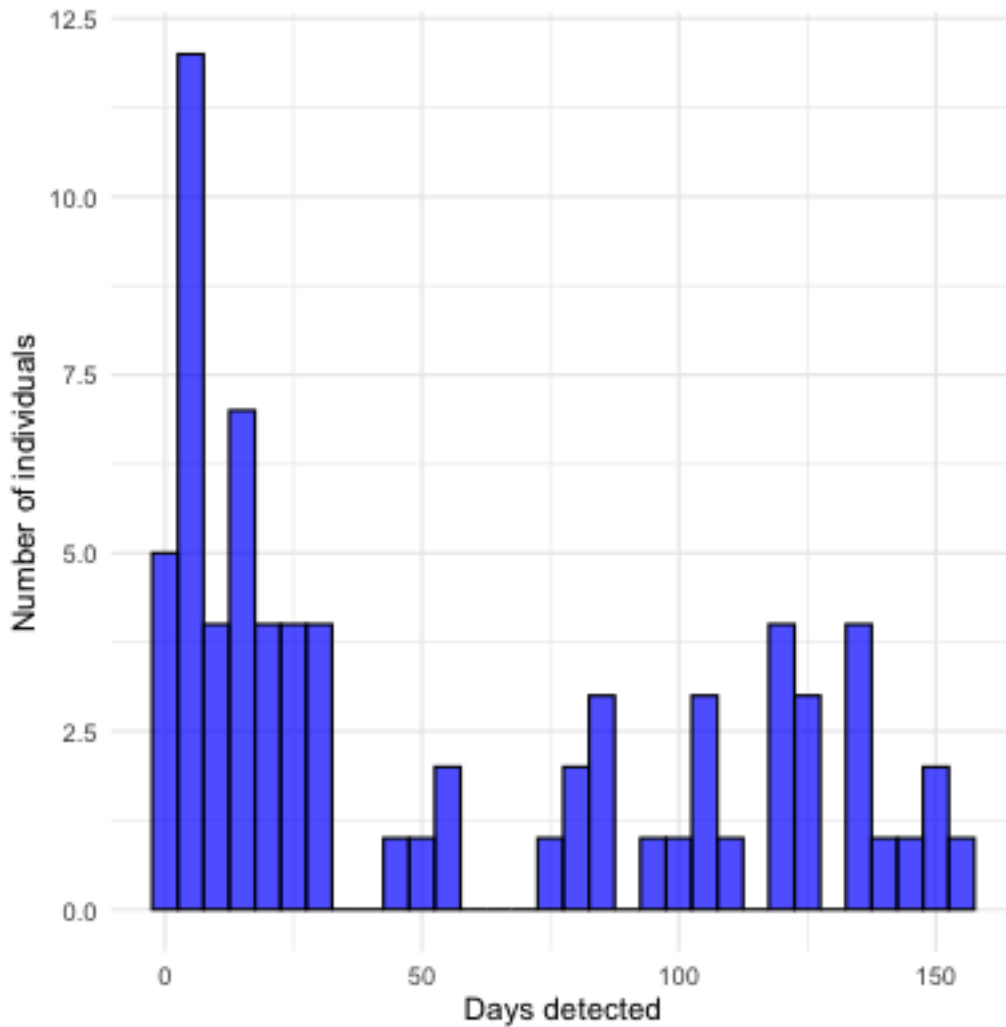


Figure 5. The frequency distribution of the total number of days that individual fish were detected over the entire study period. The plot represents only the fish included in the dataset used in the final analysis.

Between May 1, 2023 and October 5, 2023, there were 17,311 movements between the outfall, upstream, and downstream sites in the array. Bream made the highest number of transitions between sites while zander made the fewest (Table 2).

Table 2. The number of transitions between sites in the array per species of fish included in the dataset used in the final analysis.

Species	Transitions between sites
Bream	8,541
Pike	7,297
Rudd	1,366
Zander	107

The mean number of days of all tagged fish detected was highest at the outfall site ( $61.78 \pm 56.95$ ), followed by upstream ( $40.77 \pm 40.74$ ), and lowest downstream ( $10 \pm 14.12$ ). The minimum number of days detected ranged from 1 day downstream to 2 days at the outfall and upstream sites. The maximum numbers of days detected at each site were 51 at the downstream site, 157 at the outfall, and 121 at the upstream site.

### 3.2 Water temperature effects

To provide a descriptive overview of water temperature during the study, the water temperatures for each month were plotted for the whole array, regardless of site (Fig. 6).

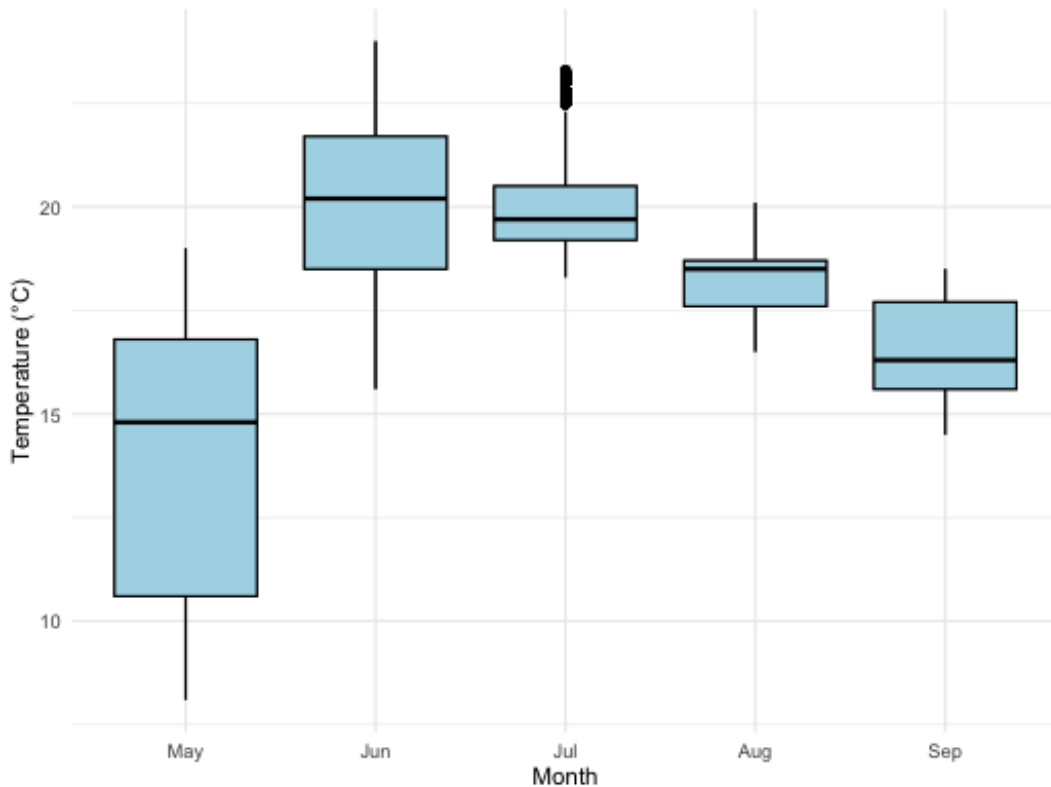


Figure 6. Water temperatures (°C) for each month. Boxplots show the interquartile range of daily water temperatures. The median lines indicate the median water temperatures of each month. The top edge of each box indicates the 75th percentile and the bottom edge indicates the 25th percentile. The whiskers extend to the data points that are within 1.5 times the interquartile range. Data points outside of this range are marked as individual dots.

To further describe the water temperatures observed in the study, the mean water temperature of each month was calculated. May had the lowest mean water temperature ( $13.9 \pm 3.26^{\circ}\text{C}$ ), while June had the highest mean water temperature

( $20.0 \pm 2.01^\circ\text{C}$ ). July ( $19.9 \pm 0.92^\circ\text{C}$ ), August ( $18.2 \pm 0.74^\circ\text{C}$ ), and September ( $16.5 \pm 1.13^\circ\text{C}$ ) exhibited intermediate mean water temperature values. Next, the daily water temperature range was also calculated (Fig. 7).

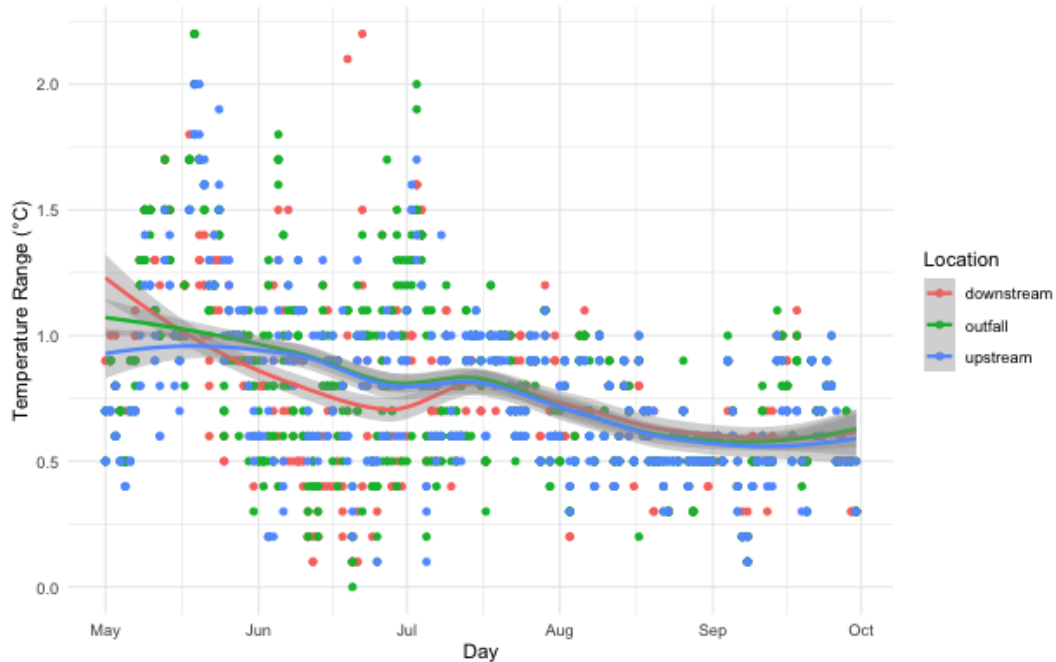


Figure 7. Daily water temperature range ( $^\circ\text{C}$ ) per site. Data points show raw values, while the smooth lines show average loess regression curves for each site. The shaded area around each curve shows the 95% confidence interval around the mean.

When we evaluated whether mean water temperature ranges varied between sites, we found that site was a significant predictor of the mean water temperature range only in June. In June, the downstream site exhibited a smaller mean water temperature range than both the outfall (GLMM,  $N = 390$ , estimate  $\pm$  SE =  $-0.14 \pm 0.04$ ,  $t(385) = -3.51$ ,  $p = 0.0014$ ) and upstream sites (estimate  $\pm$  SE =  $-0.12 \pm 0.05$ ,  $t(385) = -2.43$ ,  $p = 0.0416$ ). During the remaining months, the mean water temperature ranges did not differ between sites (all contrasts,  $p > 0.32$ ) While temperature ranges appear to differ per location in May, there is not a statistically significant variation in temperature range per location. This may be because the average marginal means are calculated for the whole month of May, therefore any significant differences that may exist only during certain days may be obscured when averaged over the whole month.

### 3.3 Weighted residency index and time spent across sites

When we assessed how weighted RI was affected by species, site, and month, we found a significant three-way interaction (GLMM,  $N = 4590$ ,  $LRT = 49.68$ ,  $p = 0.007$ ). To break down this interaction, we conducted post hoc tests within each month. Here, we compared the average weighted RI across sites within each species (Fig. 8) and vice versa (across species within each site).

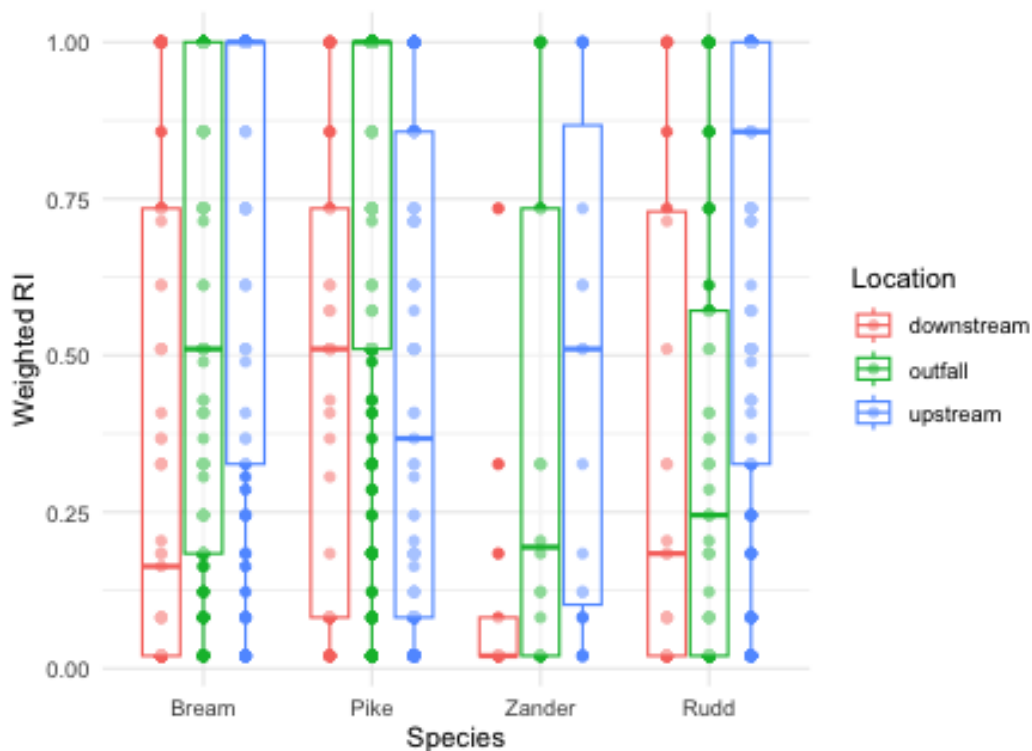


Figure 8. The weekly weighted RI of each species, split by site. Data points show the raw weighted RI values and boxplots show the interquartile range of weighted RI values. The median lines indicate median weighted RI values. The top edge of each box indicates the 75th percentile and the bottom edge indicates the 25th percentile. The whiskers extend to data points that are within 1.5 times the interquartile range. The plot represents only the fish included in the dataset used in the final analysis.

Starting first within species, pairwise comparisons showed that pike consistently showed significantly higher weekly weighted RI values at the outfall than at both the downstream and upstream sites during every month of the study period (Table 3). Rudd had significantly higher weekly weighted RI values at the upstream site than at the downstream site for every month of the study period (Table 3). Rudd also had significantly higher weekly weighted RI values at the upstream site than at the outfall in May, June, July, and September (Table 3). The weighted RI values of bream were significantly lower at the downstream site than at the outfall in July, August, and September (Table 3). Likewise, while zander showed a significantly higher weighted RI value at the upstream site than downstream in May, their weighted RI values were not significantly higher at one site compared to all other sites (Table 3).

Table 3. The results of pairwise contrasts comparing the weighted RI values between sites each month. Significant pair comparisons are indicated in red.

Month	Species	Contrast	Estimate	SE	z.ratio	p
May	Bream	downstream - outfall	-0.34	0.25	-1.387	0.3478
		downstream - upstream	-0.17	0.28	-0.612	0.8133
		outfall - upstream	0.17	0.27	0.627	0.8051
	Pike	downstream - outfall	-2.11	0.29	-7.199	<0.0001
		downstream - upstream	-1.64	0.29	-5.722	<0.0001
		outfall - upstream	0.47	0.19	2.449	0.0381
	Zander	downstream - outfall	-0.45	0.41	-1.095	0.5172
		downstream - upstream	-0.99	0.41	-2.379	0.0457
		outfall - upstream	-0.53	0.41	-1.316	0.3864
	Rudd	downstream - outfall	-0.68	0.46	-1.472	0.3043
		downstream - upstream	-2.08	0.45	-4.616	<0.0001
		outfall - upstream	-1.40	0.38	-3.666	0.0007
June	Bream	downstream - outfall	-0.06	0.33	-0.165	0.9851
		downstream - upstream	-0.18	0.37	-0.47	0.8853
		outfall - upstream	-0.12	0.35	-0.341	0.9381
	Pike	downstream - outfall	-2.52	0.35	-7.201	<0.0001
		downstream - upstream	-1.02	0.34	-3.026	0.007
		outfall - upstream	1.50	0.27	5.48	<0.0001
	Zander	downstream - outfall	0.00	1.08	0	1
		downstream - upstream	0.00	1.08	0	1
		outfall - upstream	0.00	1.08	0	1
	Rudd	downstream - outfall	-0.69	0.48	-1.444	0.3185
		downstream - upstream	-1.90	0.49	-3.905	0.0003
		outfall - upstream	-1.21	0.37	-3.24	0.0034
July	Bream	downstream - outfall	-1.75	0.49	-3.58	0.001
		downstream - upstream	-0.84	0.49	-1.717	0.1986
		outfall - upstream	0.91	0.43	2.115	0.0869
	Pike	downstream - outfall	-2.34	0.34	-6.881	<0.0001
		downstream - upstream	0.06	0.36	0.161	0.9858
		outfall - upstream	2.40	0.34	7.024	<0.0001
	Rudd	downstream - outfall	-1.09	0.41	-2.666	0.021
		downstream - upstream	-2.32	0.46	-5.087	<0.0001
		outfall - upstream	-1.23	0.38	-3.196	0.004
August	Bream	downstream - outfall	-1.09	0.40	-2.751	0.0164
		downstream - upstream	-1.69	0.42	-4.055	<0.0001
		outfall - upstream	-0.61	0.34	-1.806	0.1677
	Pike	downstream - outfall	-2.79	0.44	-6.349	<0.0001

		downstream - upstream	-0.83	0.47	-1.764	0.1818
		outfall - upstream	1.96	0.32	6.194	<0.0001
	Rudd	downstream - outfall	-0.43	0.39	-1.121	0.501
		downstream - upstream	-1.01	0.37	-2.72	0.0179
		outfall - upstream	-0.58	0.30	-1.9	0.1387
September	Bream	downstream - outfall	-1.32	0.43	-3.067	0.0061
		downstream - upstream	-1.92	0.49	-3.903	0.0003
		outfall - upstream	-0.60	0.40	-1.528	0.2777
	Pike	downstream - outfall	-3.08	0.54	-5.691	<0.0001
		downstream - upstream	-2.08	0.54	-3.838	0.0004
		outfall - upstream	1.00	0.34	2.956	0.0088
	Rudd	downstream - outfall	-0.74	0.89	-0.822	0.6892
		downstream - upstream	-3.26	0.84	-3.869	0.0003
		outfall - upstream	-2.53	0.56	-4.502	<0.0001

Next, we compared the average weighted RI between species within each site (Fig. 9).

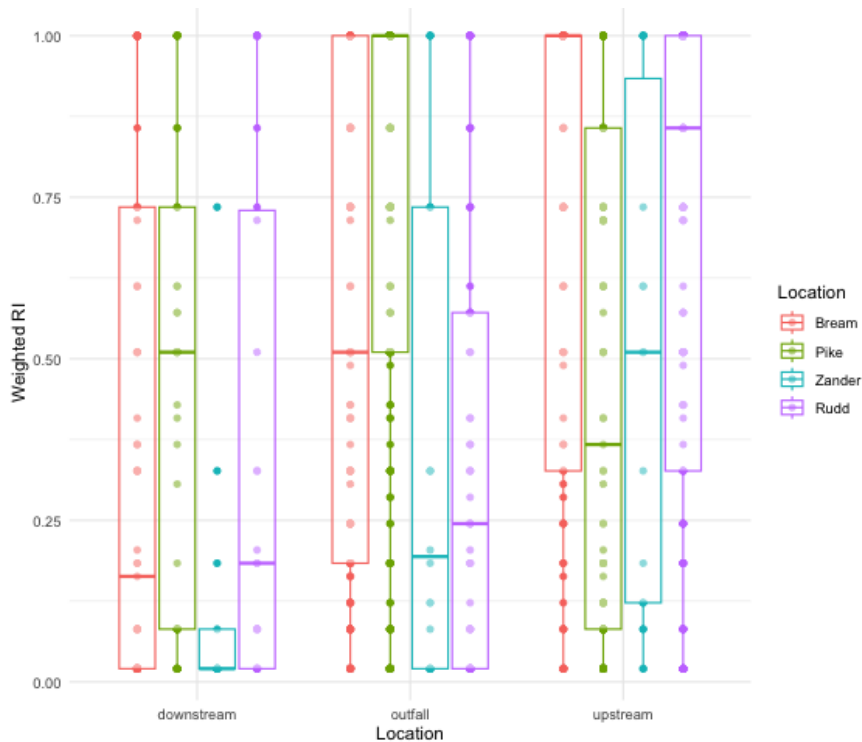


Figure 9. Boxplots show the interquartile range of the weighted RI values of each species per site. The median lines indicate the median weighted RI values of each species per site. The top edge of each box indicates the 75th percentile and the bottom edge indicates the 25th percentile. The whiskers extend to the data points that are within 1.5 times the interquartile range. Data points outside of this range are marked as individual dots. This plot represents only the fish included in the dataset used in the final analysis.

Within each site, pairwise comparisons showed a difference in mean weekly weighted RI values across all 4 species. In May, June, July, and August, bream had significantly lower weighted RI values than rudd at the upstream site (Table 4). In addition, in May, June, July, and August, bream had significantly lower weighted RI values than pike at the outfall (Table 4). Rudd demonstrated a non-significant trend of higher weekly weighted RI values than other species at the upstream site in May, June, July, and August (Table 4).

Table 4. The results of pairwise contrasts comparing the weighted RI values of species at each site during each month. Significant pair comparisons are indicated in red.

Month	Site	Contrast	Estimate	SE	z-ratio	p
May	Downstream	Bream - Pike	0.58	0.36	1.61	0.3742
		Bream - Zander	0.19	0.43	0.44	0.9718
		Bream - Rudd	-0.10	0.49	-0.20	0.9972
		Pike - Zander	-0.39	0.46	-0.85	0.8313
		Pike - Rudd	-0.68	0.51	-1.33	0.5459
		Zander - Rudd	-0.29	0.57	-0.51	0.9577
	Outfall	Bream - Pike	-1.18	0.29	-4.02	<b>0.0003</b>
		Bream - Zander	0.08	0.42	0.19	0.9976
		Bream - Rudd	-0.43	0.42	-1.02	0.736
		Pike - Zander	1.26	0.41	3.11	<b>0.0103</b>
		Pike - Rudd	0.75	0.40	1.86	0.2452
		Zander - Rudd	-0.51	0.51	-1.01	0.7434
	Upstream	Bream - Pike	-0.89	0.31	-2.87	<b>0.0212</b>
		Bream - Zander	-0.63	0.44	-1.44	0.4768
		Bream - Rudd	-2.01	0.43	-4.70	<b>&lt;0.0001</b>
		Pike - Zander	0.26	0.40	0.66	0.913
		Pike - Rudd	-1.12	0.38	-2.97	<b>0.016</b>
		Zander - Rudd	-1.38	0.49	-2.80	<b>0.0265</b>
June	Downstream	Bream - Pike	-0.23	0.78	-0.29	0.9917
		Bream - Zander	2.17	1.42	1.53	0.4183
		Bream - Rudd	-1.64	1.08	-1.51	0.4295
		Pike - Zander	2.40	1.42	1.69	0.3316
		Pike - Rudd	-1.41	1.02	-1.38	0.5125
		Zander - Rudd	-3.81	1.61	-2.36	0.0852

	Outfall	Bream - Pike	-2.69	0.76	-3.54	0.0023
		Bream - Zander	2.23	1.42	1.57	0.3943
		Bream - Rudd	-2.27	1.04	-2.19	0.1258
		Pike - Zander	4.92	1.41	3.48	0.0029
		Pike - Rudd	0.42	0.95	0.44	0.9712
		Zander - Rudd	-4.50	1.59	-2.83	0.0238
	Upstream	Bream - Pike	-1.07	0.76	-1.40	0.4993
		Bream - Zander	2.35	1.42	1.65	0.3496
		Bream - Rudd	-3.36	1.04	-3.23	0.0068
		Pike - Zander	3.42	1.41	2.42	0.073
		Pike - Rudd	-2.30	0.95	-2.42	0.0732
		Zander - Rudd	-5.71	1.59	-3.60	0.0018
July	Downstream	Bream - Pike	-2.42	1.10	-2.20	0.0706
		Bream - Rudd	-3.99	1.50	-2.67	0.0208
		Pike - Rudd	-1.57	1.31	-1.20	0.4548
	Outfall	Bream - Pike	-3.01	1.03	-2.93	0.0095
		Bream - Rudd	-3.33	1.43	-2.34	0.0508
		Pike - Rudd	-0.32	1.27	-0.25	0.965
	Upstream	Bream - Pike	-1.52	1.07	-1.43	0.3277
		Bream - Rudd	-5.47	1.46	-3.75	0.0005
		Pike - Rudd	-3.95	1.30	-3.05	0.0065
August	Downstream	Bream - Pike	-0.64	0.90	-0.71	0.7553
		Bream - Rudd	-3.41	1.14	-3.00	0.0075
		Pike - Rudd	-2.77	1.07	-2.59	0.0263
	Outfall	Bream - Pike	-2.34	0.79	-2.97	0.0084
		Bream - Rudd	-2.76	1.09	-2.54	0.0297
		Pike - Rudd	-0.42	0.98	-0.43	0.9039
	Upstream	Bream - Pike	0.23	0.80	0.28	0.9572
		Bream - Rudd	-2.73	1.07	-2.55	0.0292
		Pike - Rudd	-2.95	1.01	-2.93	0.0095
September	Downstream	Bream - Pike	-0.48	1.09	-0.44	0.8991
		Bream - Rudd	-1.28	1.55	-0.83	0.6866
		Pike - Rudd	-0.80	1.51	-0.53	0.8566
	Outfall	Bream - Pike	-2.24	0.96	-2.32	0.0525
		Bream - Rudd	-0.70	1.39	-0.50	0.871
		Pike - Rudd	1.54	1.30	1.18	0.4628
	Upstream	Bream - Pike	-0.63	0.96	-0.66	0.7862
		Bream - Rudd	-2.62	1.33	-1.97	0.1209
		Pike - Rudd	-1.98	1.26	-1.58	0.2551

### 3.4 Detection efficiency

While the receivers at the outfall each had varying detection efficiency values (ranging from detecting between 49% - 100% of expected detections), the probability of a reference tag being detected by any outfall receiver in the array on any given day was 100% due to redundancy in the calculation of presence. Receivers 2, 6, and 9 detected up to 100% of expected transmissions from reference tags while receiver 5 detected up to 95% of expected transmissions. However, receiver 1 did not hear more than 50% of transmissions from the reference tags at any point. Over the study period, receiver detection performance fluctuated, with a decrease in June for all receivers, but particularly for receiver 1 (Fig. 10).

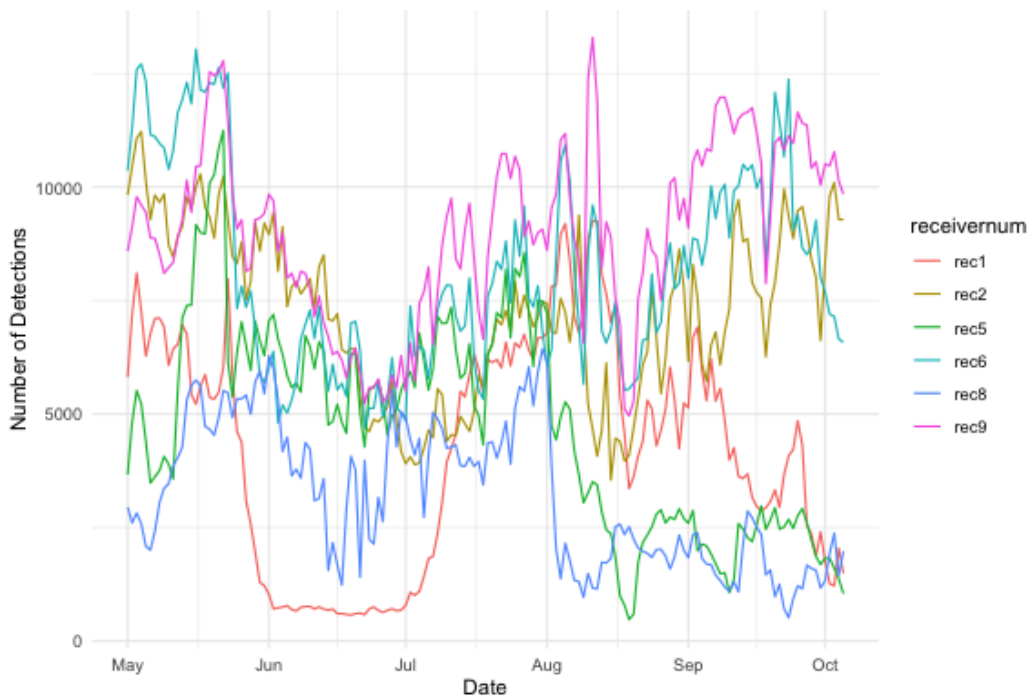


Figure 10. The number of detections per outfall receiver of transmission from the reference tags.

## 4. Discussion

Here, we studied whether proximity to a WWTP outfall impacted fish residency and water temperature ranges. Starting with the temperature ranges, I predicted that the mean water temperature ranges of each month would be greatest at the upstream site, where the thermal regulation of wastewater effluent is less pronounced. However, we found that there were no significant differences in mean water temperature ranges between the sites except during June when the downstream site exhibited a smaller mean water temperature range compared to other sites. A potential explanation for the site-specific water temperature range differences in June is that the receiving water temperatures in June may have been higher than the temperature of the WWTP effluent, which may have emphasized the cooling effect of the effluent. In contrast, during the other months, the water temperature may have been closer to the effluent temperature or the thermal impact of the effluent may have been diluted by other water sources contributing to Arboga River, resulting in no significant site-specific differences in the water temperature ranges. A possible explanation for the reduced variability in the mean water temperature range at the downstream site compared to the other sites is the increased potential for thermal mixing as the water moves away from the outfall, reducing the temperature difference between the receiving environment and the often warmer and thermally consistent WWTP effluent (Cooke et al., 2004; Mehdi et al., 2019). Additionally, due to the direction of water flow, upstream areas are less likely to be affected by the thermal properties of the effluent entering at the outfall, making the upstream site more likely to maintain greater temperature variations than the downstream site. The observation that mean water temperature range differences differ by site in June is consistent with prior research reporting that wastewater discharge can alter the thermal profiles of receiving environments differently based on their proximity to WWTP outfalls (Cooke et al., 2004; Kinouchi et al., 2007; Mehdi et al., 2021). Despite this, Arboga River is at a different latitude and continent from the locations of these studies and therefore may have differences in thermodynamic processes.

There is also a possibility that the correlation between the site and the mean water temperature range in June may be due to measurement error. Although we found a significant difference in mean water temperature ranges between sites in June, the variations were  $0.14^{\circ}\text{C}$  between the downstream site and the outfall, and by  $0.12^{\circ}\text{C}$  between the downstream site and the upstream site. Given these minor differences, it is worth investigating whether such small temperature range variations could impact fish behavior and residency patterns. Despite the lack of site-specific mean water temperature ranges in May, July, August, and September, weighted RI values still varied between sites during those

months. This suggests that the mean water temperature range is unlikely to be the primary factor influencing habitat choice in this system during those months. This notion contrasts previous studies linking water temperature to fish presence (Cooke et al., 2004; Cooke & McKinley 1999; Kessel et al., 2016; Neill & Magnuson, 1974), which further supports the idea that the low variation in temperature ranges measured in our study in June may not have been of biological significance to the fish.

In the portion of this study investigating weighted RI, I expected that fish residency would be higher at the outfall regardless of species because the fish would be attracted to the conditions created by the wastewater effluent. However, we found that the attraction to the outfall varied by species. Pike demonstrated a significant preference for the outfall over other sites during all months of the study period. This may be attributed to a concentration of prey fish, drawn to the outfall for the organic matter there. Additionally, the outfall may support more submerged vegetation around its structure, which is known to enhance residency periods for *E. lucius* (Koed et al., 2006). As a species that can adapt to a variety of environmental conditions (Casselman & Lewis, 1996), pike may also be more tolerant to WWTP effluent than other species, allowing it to benefit from some of the conditions at the outfall.

Bream showed a preference for the outfall compared to the downstream site in July, August, and September, which could be due to the avoidance of predation risk from pike (Eklöv & Hamrin, 1989; Nilsson & Brönmark, 2000; Yazicioglu et al., 2018) or a lack of preferred environmental conditions, such as muddy bottoms (Prchalová et al., 2008). In May, June, July, and August, bream had significantly lower weighted RI values than rudd at the upstream site. In the same months, bream also had significantly lower weighted RI values than pike at the outfall. A potential explanation for these disparities may be seasonal shifts in habitat use by bream. Bream migrate during the spring spawning season, entering shallow tributaries during spring when temperatures rise, then into deeper tributaries with slower flows during the autumn and winter (Gardner et al., 2013; Lucas & Baras, 2001), although individuals have shown varying migratory and residency behaviors (Schulz & Berg, 1987, Brodersen et al., 2019). These migrations may also explain why bream had more inter-site transitions than other species.

Rudd showed a significant preference for the upstream site over all other sites in May, June, July, and September, and a significant preference for the upstream site over the downstream site across all months of the study period. While rudd would likely feed on organic matter from wastewater effluent, it may have preferred the upstream site to avoid predation risk from pike at the outfall (Bean & Winfield, 1995). Finally, zander migrates between spawning, feeding, and wintering areas (Lehtonen & Toivonen, 1988; Sonesten, 1991; Nyberg et al. 1996). Indeed, it is known that zander migrate up

Arboga River and into a river near Lake Mälaren to spawn in the spring season before returning to Lake Mälaren (Lappalainen et al., 2003; Svärdson & Molin 1973), which likely explains the absence of zander individuals after June 4, 2023.

This study on the impact of WWTP effluents on the residency patterns of pike, bream, zander, and rudd can support efforts to secure funding for fish conservation and monitoring, as well as to mitigate wastewater impacts on river systems. The observed preferences of pike for WWTP outfalls and rudd for upstream areas can guide species-specific management strategies for populations found near WWTPs. For example, efforts could focus on deterring pike from congregating at WWTP outfalls and maintaining upstream areas free of pollutants to benefit rudd populations. Understanding how pollutants influence fish spatial use is crucial for effective management, especially with climate change increasing the toxicity of some pollutants (Ficke et al., 2007; Morrongiello et al., 2011; Pletterbauer et al., 2015; Vörösmarty et al., 2000). Despite high wastewater treatment rates in Sweden (96% treated biologically and chemically; Naturvårdsverket & SCB, 2022; SCB, 2018), WWTP effluent still contributes various pollutants in Sweden (Paxéus, 2004; Lundqvist et al., 2019; Söregård et al., 2019; Svenson et al., 2003; Undeman et al., 2022). To my knowledge, this study is the first to analyze the impacts of WWTP effluent exposure on fish residency patterns in situ in Sweden, offering new insights into these effects at northern latitudes.

## 4.1 Research qualifiers

Signs of fishing or predation can be overlooked in detections; for example, a moving predator could be misidentified as a tagged fish (Gibson et al. 2015; Jepsen et al. 1998; Thompson et al. 2015). Similarly, a dead, tagged fish transported by currents might be recorded as a living fish (Muhametsafina et al., 2014). While changes in detection patterns due to a fish being fished or predated on can sometimes be observed in abacus plots (Kraft et al., 2023), such scenarios may still go unrecognized, potentially leading to inflated weighted RI values.

Receiver detection efficiency decreased in June, particularly for receiver 1, suggesting that an environmental factor affected all receivers similarly during this period. Environmental factors such as wind, streamflow turbulence, turbidity, or noise may have reduced detection efficiency (Cooke et al., 2013; Clements et al., 2005; Heupel et al., 2006; Hobday & Pincock 2012; Heupel & Webber, 2012; Kessel et al., 2014a; Steel et al. 2014; Stokesbury et al. 2016; Voegeli et al., 1998). Physical obstructions, such as rocks, aquatic vegetation, or biofouling on the receivers could also interfere with transmissions (Heupel et al., 2008; Simpfendorfer et al., 2002). Despite the lower detection efficiency of receiver 1, it is

unlikely to have affected the weighted RI values since all the receivers at a given site were treated as a single unit for calculating weighted RI values for each site, thus incorporating redundancy into the calculation method.

When analyzing the relationship between mean water temperature range and site, generalized additive models (GAMMs) provide advantages over GLMMs. GAMMs can handle temporal correlations and non-linear relationships with more flexibility and without the risk of overfitting (Wood, 2017). For simplicity, GLMMs were applied to each month's dataset separately, with diagnostic plots used to address model assumptions such as stationarity, homoscedasticity, and collinearity. However, this approach may exclude some insights that GAMMs could provide by analyzing mean water temperature range and site over time.

## 5. Conclusion

By using acoustic telemetry to examine the residency patterns of pike, bream, rudd, and zander with their proximity to the Arboga WWTP outfall, we found that pike exhibited a significant preference for the outfall, while rudd showed a significant preference for upstream sites. A relationship between site and water temperature range was evident only in June, suggesting that for other months of the study period, water temperature range is unlikely to be a primary influence on fish habitat choice.

This study highlights the need for future research on potential seasonal variations in water temperature ranges near WWTP outfalls and their effects on fish. Future investigations are encouraged to focus on transitional months with large water temperature changes and explore other factors influencing fish residency patterns in this river system, as water temperature alone is unlikely to be the primary influence. Given that the lower portion of the Arboga River contains spawning grounds for various fish species (Pettersen, 2009) and that the impacts of wastewater exposure on fish reproduction are well-documented (Bahamonde et al., 2015; Bjerselius, 2001; Fuzzen et al., 2015; Hammerschmidt et al., 2002; Harris et al., 2011; Howell et al., 1980; Jobling et al., 1998; Lange et al., 2011; Nash et al., 2004; Oshima et al., 2003; Parrot & Blunt, 2005; Saaristo et al., 2014; Schoenfuss et al., 2002), future studies should examine whether Arboga WWTP effluent affects fish spawning behavior further downstream. These findings enhance our understanding of the impact of chemical releases on aquatic environments, which is particularly relevant as the human population grows (UN Department of Economic and Social Affairs, 2017). RI can serve as an indicator of how fish respond to pollution in the natural environment, which may be useful as governing bodies continue to implement and modify regulations on river pollution. Researching fish habitat choice in natural environments rather than in controlled laboratory settings incorporates the dynamics of aquatic ecosystems and can therefore offer a more accurate representation of fish behavior.

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## Popular science summary

As our world becomes more crowded, more pollutants are released into the environment<sup>1,2</sup>. One way these pollutants enter our waterways is through wastewater treatment plants (WWTPs), which clean and treat wastewater before releasing it back into rivers and lakes. But disappointingly, while WWTPs remove a portion of pollutants, they don't get rid of all of them. The remaining pollutants that make it into our waterways can affect the fish that live there, changing their behavior and health<sup>4,5</sup>. Some of these behavioral changes can even influence how likely it is that a fish will survive<sup>6</sup>, so it's not an exaggeration to say that pollutants can harm fish populations<sup>7</sup>. Despite the higher concentrations of certain pollutants at outfalls, where the treated water enters the waterway, fish are still found living near outfalls<sup>8,9,10</sup>. Why? Scientists think fish might like the warmer or more stable temperatures of the wastewater, and that fish be drawn to outfalls for the buffet of nutrients found in the wastewater<sup>11,12,13,14</sup>.

For the first time in a natural setting in Sweden, we looked at how wastewater affects where fish go and for how long. From spring to fall of 2023, we tagged 80 fish of varying species in Arboga River in southern Sweden so they could track their movements around a WWTP outfall. The tags they used work like the trackers you might use on your keys or in your luggage. The devices send out pings to show where fish are. Other devices hear the pings and record the ID of the fish, along with its location and the time. This allowed us to track where the fish spent their time (upstream, downstream, or at the outfall) and how long the fish stayed in each area.

What they found was surprising: even though wastewater is often warmer than the water it flows into<sup>15</sup>, during most months, this wastewater didn't cause larger temperature changes in different areas of the river. Despite this, fish still had distinct preferences for where they liked to stay and it didn't seem to be because of water temperature. For example, pike spent most of its time at the outfall, possibly for the prey or the shelter there, while rudd preferred the upstream area, perhaps avoiding being eaten by the pike. Each species studied had different preferences for where it liked to stay and for how long.

These findings are just the beginning, though. We still have much more to learn about where fish prefer to stay around WWTP outfalls. This is especially important because climate change can cause some pollutants to become even more harmful to organisms<sup>16</sup>. As pollution from human activity continues to enter our waterways, we should learn all we can about how fish interact with the pollutants so we can better protect our fish species. Given Sweden's northern latitude, we can use these findings to shape laws and practices around wastewater management to better protect fish populations in other northern parts of the world.

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# Acknowledgements

Thank you to my supervisors Erin McCallum and Natalia Sandoval Herrera for giving me such generous help in writing this thesis.