The Influence of Metabolic Performance on Pharmaceutical Bioconcentration in Brown trout (*Salmo trutta*)

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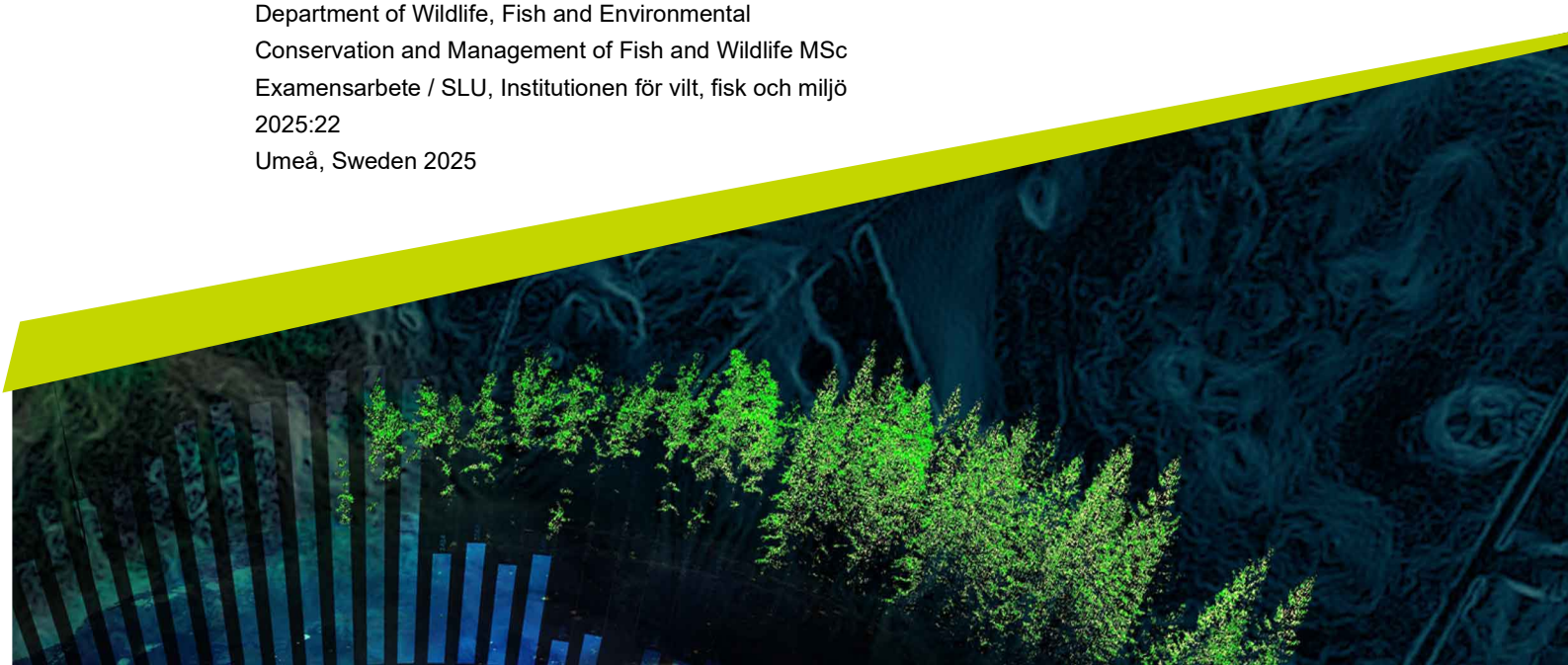

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Keywords:	intermittent respirometry, metabolic performance, oxazepam, fish, pharmaceutical contaminants, bioconcentration

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Abstract

The increasing global detection of pharmaceutical contaminants in aquatic ecosystems is of significant environmental concern. Especially concerning are pharmaceuticals that affect non target species like fish through bioconcentration of pharmaceutical contaminants, which can have severe ecological consequences. Ecotoxicological studies are increasing but few studies have focused on what factors determine bioconcentration of pharmaceuticals in fish tissues. Metabolic performance plays a vital role in essential functions such as growth, reproduction, and movement and is hypothesized to play an important role in the intake and further bioaccumulation of contaminants. This study aimed to examine the effect of metabolic performance on the bioconcentration of the pharmaceutical oxazepam, comparing concentrations during two different exposure times. Additionally, I looked at possible predictors of metabolic performance in brown trout (*Salmo trutta*) from two different hatchery strains. Their metabolic performance was assessed in an intermittent respirometry setup by measuring their standard metabolic rate (SMR), maximum metabolic rate (MMR) and absolute aerobic scope (AAS). Afterwards the fish were exposed to an oxazepam concentration of 18 µg/L for a short period of 24 hours and a longer one of seven days. The results revealed a significantly higher concentration of oxazepam in trout's blood plasma during the exposure time of seven days as well as a significantly higher concentration for fish showing increased metabolic performance. The results also showed that length was a predictor of all metabolic measurement AAS, SMR and MMR but not exhaustion time. The results highlight the significance of studying the mechanisms and factors driving bioconcentration of pharmaceuticals in fish, including the role of metabolic performance for predicting the movement of contaminants through aquatic food chains, evaluating long-term ecological risks and implementation of conservation management strategies, especially as pharmaceutical consumption continues to rise worldwide.

Keywords: intermittent respirometry, metabolic performance, oxazepam, fish, pharmaceutical contaminants, bioconcentration

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Abbreviations

Abbreviation	Description
AAS	Absolute aerobic scope
MMR	Maximum metabolic rate
MO_2	Metabolic oxygen consumption
N	Hatchery in Norrfors
S	Hatchery in Skellefteå
SMR	Standard metabolic rate

1.Introduction

The increasing global consumption of pharmaceuticals has become a significant environmental concern, particularly for aquatic ecosystems (Deblonde et al. 2011; Arnold et al. 2014; Aus Der Beek et al. 2015; Brodin et al. 2017). A wide range of human and veterinary pharmaceutical residuals, including steroid-hormones (Bertram et al. 2018), analgesics, antibiotics, antidepressants and sedatives, are frequently detected in freshwater and marine environments as well as in surface waters (Brodin et al. 2014; Heynen et al. 2016a; Hellström et al. 2020). This contamination occurs through pathways such as treated and untreated sewage, wastewater treatment plants that lack adequate filtration, pharmaceutical manufacturers and improper disposal practices (Deblonde et al. 2011; Brodin et al. 2017). As prescriptions for pharmaceuticals continue to rise, the persistence of these compounds in water systems is an escalating issue (Arnold et al. 2014; Aus Der Beek et al. 2015; Bernhardt et al. 2017; Brodin et al. 2017). The impacts of pharmaceutical contamination on aquatic life range from endocrine disruption to antimicrobial resistance (Santos et al. 2019; Ramírez-Morales et al. 2021) to behaviour alterations (McCallum et al. 2021) and often affects non-target species, which can have severe ecological consequences. For instance, a study on guppies revealed that long-term exposure to pharmaceuticals can have negative impacts on sperm vitality leading to decreased reproductive ability (Aich et al. 2025).

Research on bioaccumulation of pharmaceuticals, their biotransformation and effect on behaviour and physiology is of rapidly increasing interest in recent years (Puckowski et al. 2016; Miller et al. 2018). The accumulation of these pharmaceuticals in fish can occur indirectly through dietary uptake, by consumption of exposed organisms (Heynen et al. 2016a; Bose et al. 2022; Becker et al. 2024), or directly through the surrounding water, absorbed across skin and gills (Yang et al. 2020), the latter process is called bioconcentration (Heynen et al. 2016a; Duarte et al. 2022). An increase of bioconcentration potentially lead to elevated concentration of contaminants in tissues (Wang 2016), which enables the movement of pharmaceutical compounds through the food chain (Vargas-Villalobos et al. 2024). However, the pharmaceutical concentration in aquatic environments often does not accurately reflect the concentration found within the tissues of aquatic organisms (Brodin et al. 2013, 2014; Miller et al. 2018). Concentrations of pharmaceuticals have been found to be tissue specific in fish, with lower concentrations in muscle tissue and higher concentrations in plasma (Heynen et al. 2016a; Miller et al. 2018; Cerveny et al. 2021a). Previous studies have shown that the pharmaceutical concentration of some parent compounds like e.g. temazepam, found in fish tissues and blood plasma, were lower than the concentrations of their transformed metabolites e.g. oxazepam. As

a consequence, metabolites like oxazepam are likely to be underestimated in conservation management plans, risk assessments and in their possible impacts on biota (Puckowski et al. 2016; Cerveny et al. 2021a). This highlights the limited understanding of the processes underlying bioaccumulation and emphasizes the need for further research in this area.

Bioconcentration, the absorption of waterborne chemicals (Streit 1998), is determined by multiple factors including environmental and intrinsic factors. Those parameters include environmental variables such as salinity, pH, occurrence of other contaminants, and water temperature, as well as intrinsic factors like species, age, size and weight, sex, behaviour, fat content, and metabolism (Brodin et al. 2013; Heynen et al. 2016b; McCallum et al. 2019; Cerveny et al. 2021b; Duarte et al. 2022). For example, in a study on European perch (*Perca fluviatilis*) and dragonfly larvae (*Sympetrum sp.)*, their exposure to temazepam, a commonly prescribed benzodiazepine to treat insomnia, revealed that the bioconcentration and biotransformation of pharmaceutical compounds like temazepam and its metabolite oxazepam were temperature dependent and species specific. While the dragonfly larvae showed no temperature dependent bioconcentration, the bioconcentration in perch showed a strong temperature dependency, as well as the biotransformation in its metabolite oxazepam (Cerveny et al. 2021b). There are few studies on how factors e.g. temperature, species, body size and pharmaceutical mixtures affects the uptake of pharmaceuticals in fish, but one unexplored aspect is an individual's physiological phenotype, including metabolic performance.

Metabolic performance, the capacity of an organism to convert energy into physiological processes, is the engine behind many physiological traits like growth, reproduction, foraging and digestion (Fu et al. 2009; Archer et al. 2021). These physiological traits form the foundation for specific life history strategies, behaviours and adaptations to environmental changes (Finstad et al. 2007), for example pharmaceutical contamination. Research on metabolic performance on fish have a longstanding history (Brett 1964; Fry 1971) and since the technological advancements are increasing, advanced insights into physiological importance are feasible (Metcalfe et al. 2016). Metabolism plays a key role in uptake, distribution and detoxification of contaminants in fish (Du et al. 2018; Matthee et al. 2023) and impacts fish growth and survival (Allen et al. 2016), may even influence migration and movement patterns, behaviour, and even hierarchical positions (Killen et al. 2012; McCallum et al. 2021). A study on brown trout demonstrated that physiology, behaviour and social status are interacting and impacting the bioaccumulation of pharmaceuticals (McCallum et al., 2021), and thus highlighting the importance of studying the impacts on

pharmaceutical uptake and metabolic performance in fish in more detail. Measurements of metabolic performance can be assessed in several ways including intermittent respirometry. Respirometry is assessing oxygen consumption by measuring the oxygen decline of fish in a closed chamber (Svendsen et al. 2016). With this information on oxygen consumption, we can estimate indicators of metabolic performance like standard metabolic rate (SMR), maximum metabolic rate (MMR) and absolute aerobic scope (AAS) (Metcalfe et al. 2016; Norin & Clark 2016). Standard metabolic rate represents the minimum amount of energy that an organism requires for maintenance in a resting condition (Metcalfe et al. 2016) while maximum metabolic rate reflects the highest rate of oxygen uptake an organism can achieve to generate energy (Norin & Clark 2016). To achieve the upper limit of metabolic performance, it is necessary to measure oxygen consumption while the animal is an energy demanding task like swimming at high current or bursting until being exhausted (Farrell 2008; Zhang et al. 2020). After the fish is exhausted, MMR is measured as a steep oxygen decline. In combining both measures, the "metabolic floor" and the "metabolic ceiling" of an organism, AAS captures a good representation of an organism's capacity to perform and cope with its environment. (Fu et al. 2022). As AAS is influenced by environmental changes, such as pharmaceutical pollution it is a valuable bioindicator in studies on contaminant uptake (Fry 1971; Fu et al. 2022).

The aim of this study is to investigate whether various measures of metabolic performance predict the bioconcentration of the pharmaceutical oxazepam in two different strains of brown trout over a short exposure of 24 hours and longer one of 168 hours (seven days). Additionally, I tested whether factors like body condition and size, strain, treatment group or time to exhaustion are predictors of metabolic phenotypes.

1.2 Objective

This thesis will focus on examining the bioconcentration of the pharmaceutical oxazepam in brown trout (*Salmo trutta*) and how this is influenced by fish metabolic performance. Specific objectives include:

1. Assessing MMR and swimming fatigue time through subjecting trout to a standardized exercise protocol which resulted in individual exhaustion endpoints.;
2. Assessing trout Absolute aerobic scope (AAS) as a physiological indicator of metabolic performance by measuring their standard metabolic rate (SMR) and maximum metabolic rate (MMR) through intermittent respirometry.
3. Quantifying the concentration of oxazepam in trout's blood plasma after a short exposure time 24 hours and a longer one of 168 hours (seven days).
4. Evaluating the relationship between metabolic performance as aerobic scope and the bioconcentration in plasma.

1. Hypothesis: Blood plasma concentrations of oxazepam are influenced by trout's absolute aerobic scope as a measurement of metabolic performance and its time of exposure as metabolism of both fish and pharmaceuticals influences uptake, distribution and excretion of oxazepam in fish.

For the pharmaceutical part of this study, I predict that a higher absolute aerobic scope (AAS) results in a higher concentration of oxazepam in fish blood plasma. As oxazepam is more slowly absorbed than other benzodiazepines (BZD) due to its low solubility (Dinis-Oliveira 2017) and higher metabolically active fish (high AAS) will show a higher concentration of oxazepam in their blood plasma due to faster uptake of the drug.

Several factors including strain, body condition such as size and weight, treatment and exhaustion time can affect the metabolic phenotype (including all metabolic measures of AAS, SMR, MMR, time until exhaustion) which may therefore directly or indirectly affect the uptake of pharmaceuticals of trout. Thus, I looked at possible predictors of metabolic performance. Thus additional objective includes: 5. Evaluating how fat content, length, strain, treatment and exhaustion time influences metabolic performance.

2. Hypotheses: Size, weight, fat content as an indicator of body condition, exhaustion time and strains are predictors of AAS, SMR, MMR and exhaustion time.

Furthermore, I predict for the physiological part of this study that variables like length, strain, fat percentage and exhaustion time do have an impact on metabolic performance. The time a fish needs to be exhausted can be affected by fat content as shown in previous studies (Duarte et al. 2022), as increased percentage of fat provides more energy storage and therefore can prolong endurance of fish. Additionally, I predict that there is a relationship between length and weight on metabolic performance as previous studies have shown a link between length, gill surface and metabolic rates (Kuparinen et al. 2022).

2. Material and Methods

2.1 Study animal

In this study I used brown trout as a study animal which is due to its widespread global distribution, genetic diversity, and significant economic value as both a game fish and food source (McGlade et al., 2022) an ideal model species. Besides its ability to adapt across diverse environments, trout shows a wide range of behaviours and physiological traits that are interesting to study such as environmental sensitivity, migratory and territorial behaviour, and several different forms of life stages. Brown trout exhibit two life history strategies, as anadromous (Sea trout) which migrates to the sea, and residential forms which remains in freshwater rivers. Both life forms include the developmental stages of eggs, alevins, parr, smolt and adult (Jonsson & Jonsson 2009) which show varying tolerances to oxygen saturation, temperature fluctuations and environmental pollution (Elliott & Elliott 2010). This sensitivity makes it a valuable bioindicator of environmental changes and an interesting model species for studies on stress and pollution responses. Furthermore, brown trout is a well-suited due to its ecological position as predator, as a native freshwater species in Sweden and its availability as hatchery fish and as wild fish which makes it ideal to study effects in a laboratory and in a natural setting.

These characteristics and its ecological importance, elevates its relevance in environmental studies and given its broad geographic range, the brown trout is well suited for examining the effects of environmental pollution and bioconcentration across diverse ecosystems.

2.2 Experimental animals and husbandry

Juvenile brown trout (*Salmon trutta*) at the age of one to two years were transported from two different fish hatcheries in Sweden, Norrfors (N) (n=69, average size: 17.909, S.D. ±1.011), Umeå Municipality, and Skellefteå Municipality (S) (n=82, average size: 18,510, S.D. ±1,189), to aquatic research laboratory of the department of Wildlife, Fish and Environmental studies at the Swedish University of Agricultural Sciences' in Umeå. I will refer throughout the thesis to the different strains of trout coming from Norrfors as (N) and Skellefteå as (S). The fish arrived on September 16th (N) and 20th (S) 2024 and were acclimated for four weeks prior the start of the experiments for acclimation.

The fish were housed in 840 L tanks (L170xW113xH88 cm) with filtered, constantly flowing water and airlines providing steady Oxygen supply. Each strain was kept in a separate tank. To provide environmental enrichment, artificial green aquarium plants, black tunnels (PVC) and clay dens were provided. Additionally, three pumps (Eheim) were installed to create water currents, simulating their natural habitat. Half of the tank was covered with a tarp for additional shelter, and a net was covering the tanks to prevent the fish from jumping out.

The water quality was monitored twice a week initially and after 3 weeks reduced to once a week. The parameters measured included pH (7-8), dissolved oxygen (9,15 -12,5 mg/L), nitrite (NO_2) (0 mg/L), nitrate (NO_3) (0 mg/L), and water temperature (9,9 – 12,2 °C) using an optical dissolved oxygen (DO) and temperature meter (Ecoense ODO200) and water test stripes (brand).

The fish were fed daily, except for one fasting day per week to mimic natural conditions. Upon arrival, the fish were fed daily with pellets from their individual hatcheries to facilitate acclimation. Following a food transition one week after their arrival over a course of 10 days, switching all fish to a common pellets diet from the Norrfors hatchery to ensure uniform feeding conditions. The light cycle was automatically controlled, set from 08:00 to 19:00. Fish were health checked daily in their housing tanks across study period.

Upon arrival fish were measured and tagged. We first sedated the fish individually using a solution of MS-222 with buffer (concentration: 0.25 mL/L), waited until fish was unresponsive to the touch (5 sec), then measured length (fork and total), weight, and fat content using a Fatmeter (Distell Fish Fat Meter Modell-FFM-992). Finally, we inserted a RFID tag (Biomark, APT12 PIT), performing a small incision (~5mm) in the intraperitoneal area behind the pelvic fin for proper identification. Individuals that were wounded, unhealthy or were smaller than 40 g, were excluded to ensure the ideal fish chamber ratio for the respirometry study. An ideal fish chamber ratio for reliable Oxygen consumption detection reduces stress in fish due to small chamber while a too large chamber makes it hard to detect a decline in Oxygen (AutorespV3 LoligoSystems).

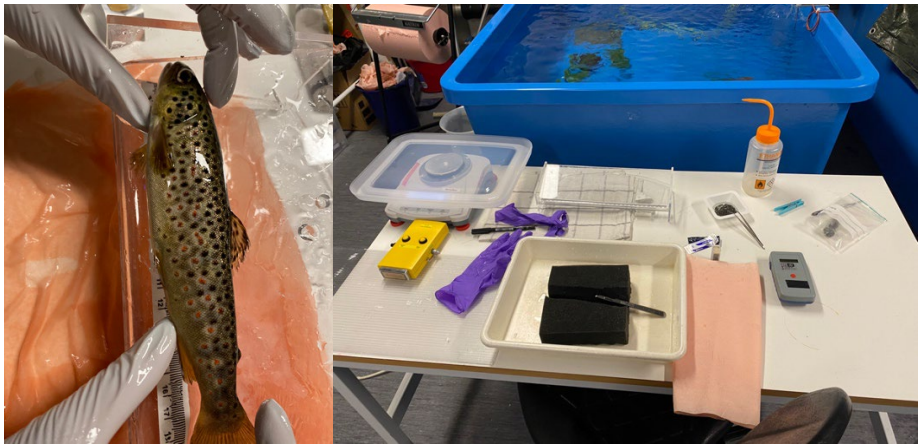

Figure 1: Brown trout sedated and measured. Equipment for measurement and PIT tagging of fish including the fatmeter.

2.3 Experimental Setup

During the experimental period we had two experimental groups, one longer with an exposure of seven days and one shorter with an exposure of 24 hours. The first experimental group included six test groups in total while the shorter exposure period included two test groups, eight test groups in total. All eight test groups consisted of eight fish each. Each group included an equal number of both strains (N, S) and exposed and control fish (Table 1).

Each day we selected eight fish randomly, selected from each strain and treatment (Table 1). At first in the morning (Figure 2), each fish was tested in a behavioral trial and afterwards underwent an exhaustion/exercise protocol to be able to measure the maximum metabolic rate described in section 2.4. After reaching the criteria for exhaustion (Farrell 2008; Norin & Clark 2016), the fish was placed in a respirometry chamber set up for a minimum 18 hours to quantify oxygen consumption described in section 2.4. After a maximum of 23 hours each fish was transferred to the exposure room and placed in individual tanks. All fish were first fed, and the individuals selected for exposure were exposed after one hour with a concentration of 18 µg/L of oxazepam. We created two exposure groups, one shorter with 24 hours (n= 16) of exposure and longer one of 168 hours (seven days, n=26) to assess time dependent accumulation of oxazepam in plasma. All individuals were dissected, and tissues were sampled after completing the exposure period of either seven days or 24 hours. The experimental timeline is shown in figure 2.

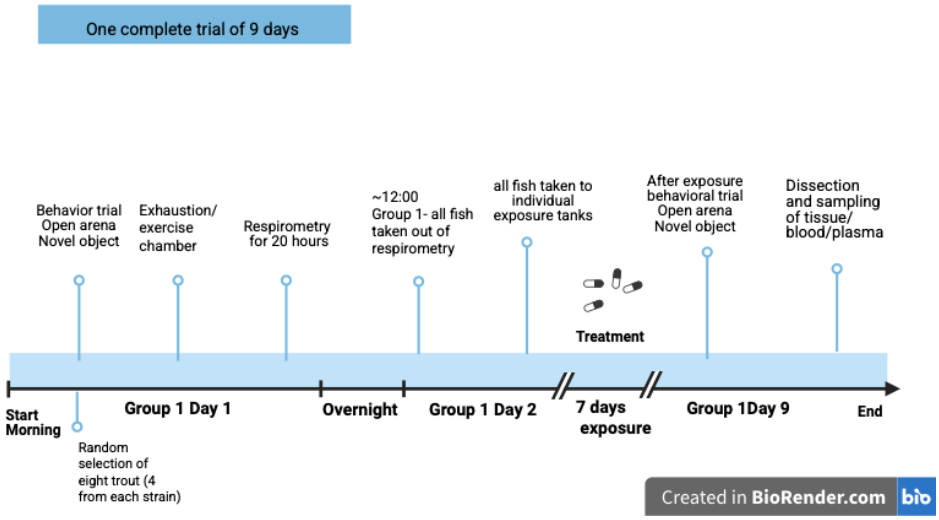

Figure 2. Timeline of experimental steps of one testing round of nine days.

2.4 Exhaustion/Exercise protocol

For the exercise/exhaustion protocol, I used a custom setup which included a tank (105x105x42) with two chambers (1600 ml volume). Each chamber was equipped with a plastic grid at one end and a water filter sponge connected to a pipe at the other. Each chamber was connected through the pipe to an Optiflow EVO 2 flow restrictor which controlled the flow in three steps (Average: 0,044; 0,65; 0,108 m/sec) and a pump (Eheim, Germany Typ 1262). Each chamber was marked with a white stripe dividing the chamber into two equal parts. After placing the fish into the chamber, the flow was increased every minute until the maximum was reached (Jain et al. 1997; Farrell 2008). The fish was considered exhausted when it fell back on the grid, behind the white mark, not moving for at least 20 seconds. Maximum trial time was 20 minutes. After exhaustion, the fish was immediately transferred to the respirometry chamber which took not more than ten seconds.

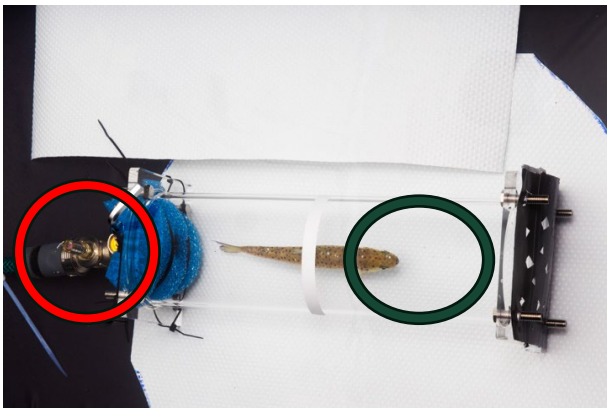

Figure 3: Picture of the custom-built exercise tunnel used for the exhaustion/exercise protocol. The flowmeter (red circle) was used to control the stepwise increase in flow. The white mark indicates the reference point, and the green circle shows the area where the fish was considered exhausted, determined as falling back onto the grid and remaining still for at least 20 seconds. (Image taken by Jörgen Wiklund).

2.5 Respirometry Setup

The respirometry set-up (LoligoSystems, Viborg, Denmark) was arranged in a separate room, regulated at a constant temperature of 10 °C. A pool, size large (Ø 160 x H 30 cm) with eight acrylic chambers (200 mm length, 1,6 L volume) and a non-gas permeable tube system (Volume =113 ml), provided the experimental setup (Figure 4). Each chamber was connected to two pumps. One pump flushed the water through the chamber to exchange deoxygenated water during the flush phase, while the second recirculation pump pushed the water through the system in order to pass the oxygen sensor which measured dissolved oxygen (mg/l) every second throughout the experiment. Additional airlines assured proper oxygen saturation during the trial period.

To ensure all fish were in a postabsorptive state, they were fasted 24 hours prior to the start of the experiments. Fish were haphazardly caught and identified by their individual tag. Each of the eight trial groups consisted of eight chambers, with one fish per chamber, and was tested in the following set up.

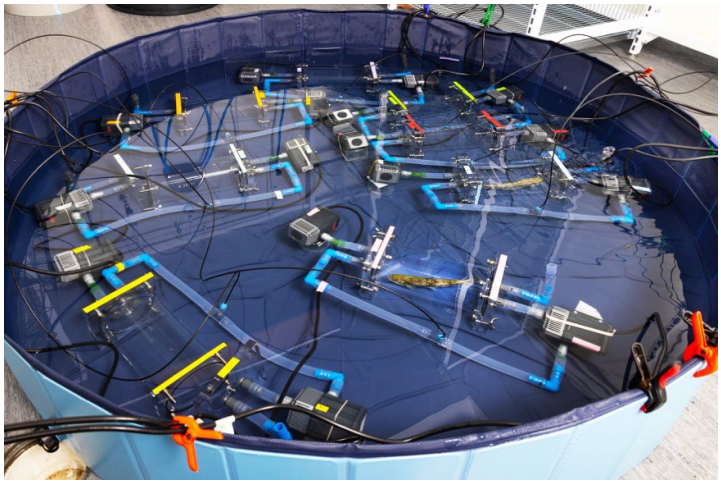

Figure 4: Experimental setup of the respirometry equipment consisting of a pool, eight chambers including eight oxygen sensors, 8 flush pumps and eight recirculation pumps, two temperature sensors all connected to the Witrox 4 devices (Image taken by Jörgen Wiklund).

Oxygen consumption (mg/kg/h) was measured using LoligoSystems, Viborg, Denmark. The system was controlled via AutoResp 3 (version 3.2.2) using intermittent respirometry. Three phases, consisting of a flush phase (240 s), a waiting period (60 s) creating a stable oxygen content in the chamber and avoiding a nonlinear oxygen curve during the following measurement phase (360 s) (Andersson et al. 2020) (LoligoSystems, 2020, figure 5). The flush pump provided oxygen-rich water which flushed through the chamber and initiated an increase of oxygen level/saturation to its baseline above 100 % air saturation. The minimum oxygen level reached through the closed phase was set to 50% but never reached below 60% (Svendsen et al. 2016).

a b

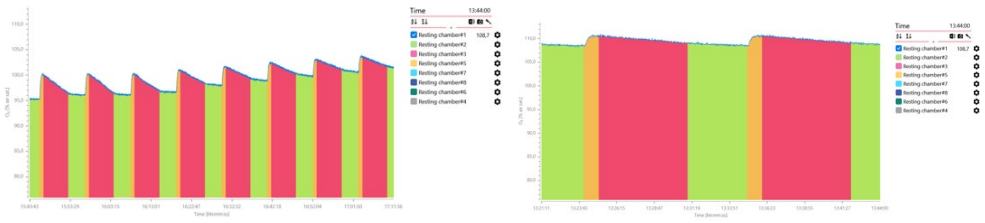

Figure 5: Shows a part of the respirometry measurements of eight (a) and two (b) loops, of which each loop consists of a flush phase in green (240 s), a waiting phase coloured in yellow (60 s), and a measuring phase in red (360 s) with a linear decline in blue.

The chambers were covered with a black plastic cover to shield fish from environmental disturbances. The room was left undisturbed after the last fish was placed in the chamber until the next morning. To account for background respiration all chambers were all run empty at least 1 hour before and 1 hour after the respirometry trials. To avoid microbial buildup in the system, we washed the whole system using a 3% household bleach and flushing the system for 1 hour between every trial.

2.6 Pharmaceutical exposure

After the respirometry experiment, fish were transferred into a separate room, housed in exposure tanks (70x50x40) separately one fish per tank, and immediately fed. Each tank was filled with 30L of water, equipped with 2 artificial plastic plants and a PVC tunnel as enrichment. Fish were kept either 7 days (Group1-5) or 24 hours (Groups 7 and 8) in the exposure room. After one hour of acclimation the tanks (30 L) that were selected as exposed, were spiked with 14 ml stock solution of oxazepam (dose: 18 µg/L). Oxazepam was prepared as a stock solution (38,3 mg/L). Unexposed individuals were left as control without exposure.

After metabolic and behavioral phenotyping, I exposed the fish to an anxiolytic drug oxazepam and conducted a second behavioral trial after the exposure period. After the exposure time, each group went through a second behavioral trial (Group 1,2,4,5,6) and were then euthanized using concentration 50 ml/L MR222 and a buffer. After euthanasia muscle, liver, brain, and heart tissues as well as blood were sampled. The blood was centrifuged for 5 minutes in order to separate the plasma from the blood. All samples were frozen in Eppendorf micro test tubes and stored until extraction and chemical analysis.

2.7 Chemical analyses

For this study, a subset of 42 plasma samples were prepared for quantification of oxazepam. We used 50 µl of plasma, which was separated in Eppendorf microtubes, and prepared in several steps for quantification with liquid chromatography mass spectrometry (LC-MS). First, 50 µl of Methanol was added to the plasma as a solvent. In the next step, an internal standard of oxazepam -d5 (IS) was added which served as a reference. In the end, 20 µl of ultrapure water (MillQ) was added and the solution was mixed using a vortex. The samples were

then mixed using a homogenizer. After 24 hours of incubation at -20C, the samples were centrifuged (10 min at 14000 rmp) and the supernatant was transferred to autosampler tubes for the final analysis of oxazepam concentration.

To quantify oxazepam in water samples from the exposure and control tanks, we collected 16 samples on 8 days and froze them at -20C for later analysis. Briefly, the water samples were thawed, and 5 ml of water was filtered through a 0.45 µM filter. Each sample was then spiked with internal standard (same as for the plasma samples), and the sample was measured directly on the liquid chromatographer mass spectrometer. For analysis of oxazepam concentration in water and blood plasma samples, the analytical methods and liquid chromatography – mass spectrometry instrumentation in (Cerveny et al. 2020) were applied.

2.8 Statistical Analyses

2.8.1 Determination and calculations of metabolic oxygen consumption (MO_2), MMR, SMR and AS

I assessed maximum metabolic rate (MMR), standard metabolic rate (SMR), and absolute aerobic scope (AAS) as an indicator of metabolic performance (Norin & Malte 2011; Archer et al. 2020).

Oxygen depletion was measured and calculated using the AutoResp v3 software (version 3.2.2, LoligoSystems) and transformed in metabolic oxygen consumption using the equation:

$$MO_2 = \frac{O2}{V*t} \text{ x } M$$

Where O2/t represents the rate of Oxygen decline (slope) in mg/l/h; V represents he chamber and tube volume minus the fish volume; and M represents the fish weight in kg.

SMR was calculated as the 10^{th} percentile of all data points for each fish using Excel. MMR was measured after the exhaustion/exercise protocol described in the section 2.3, followed by an immediate transfer to the respirometry chamber and was calculated as the average of the 3 highest values in Excel (Killen et al. 2012).

Before the calculation of SMR, MMR, and AAS loops, meaning a measurement period of flush (240 s), waiting (60 s) and measurement (360 s) (figure 5), with an estimate below a threshold of R2 below 0,9 were excluded. AAS was calculated as: AAS = MMR – SMR.

2.8.2 Statistics

All data was statistically analysed using R Studio (Version 4.4.2). To explore the effect of predictor variables on the response variable of oxazepam concentration in fish plasma, I used linear mixed effects models which included "group" as random intercept effect as shown in table 1. Predictor variables included Absolute aerobic scope (AAS), Standard metabolic rate (SMR), individual exhaustion time of each fish, exposure time (categorical: 24 hours or 168 hours (7 days)), and fish strain (categorical: Norrfors (n) or Skellefteå (s). In a separate model I included maximum metabolic rate (MMR) separately from AAS as both variables are highly correlated.

Next, I assessed the effect of strains, treatment (control/exposed), length and fat percentage on the metabolic phenotype variables absolute aerobic scope (AAS), maximum metabolic rate (MMR) and standard metabolic rate (SMR). I performed linear mixed effects models as shown in table 2. Additional I assessed another model (Appendix, table 2) which included exhaustion time as a response variable and weight as a predictor variable in addition to strains, treatment, length and fat percentage. I did not include weight in the models with the metabolic phenotype variables (AAS, SMR and MMR) as the calculations of those already included weight.

All data and models were tested for assumptions of normality using histograms, Shapiro-Wilk's test, Quantile-Quantile plots and diagnostics plots using the R package "performance". Data transformation was performed when normality was not met as in the case of oxazepam concentration with log10 for improved model fit. Furthermore, assumptions of equal variance were tested with the Levene test and diagnostics plots using the R package "performance". Further testing of the models using the diagnostic plots from the R package "performance" included normality of residuals, independence, heteroscedasticity and multicollinearity. The criteria for model selection for the pharmaceutical part of the study were based on the model with the lowest AIC (Akaike information criteria). The criteria for models chosen for the respirometry part of this study, were selected using the significant model using the likelihood ratio criteria for nested models for response variables AAS, SMR and MMR. For the model including exhaustion time as response variable, the model with the lowest AIC was chosen.

Limitations

For this study, 60 Brown trout were tested in respirometry trials of which 8 died of unknown causes and 10 were excluded because of technical issues with the chambers. Technical problems included mostly not detecting a decrease in oxygen at the beginning, or later during the experiments.

Ethical Statement

We followed all ethical statements and permissions. Experiments and animal husbandry were performed in accordance with the Ethical Committee on Animal Experiments (ethical permit number: DNR A-5-2023 from Jordbruksverket).

3. Results

3.1 Effect of metabolic performance and exposure time on oxazepam concentration

In total, 42 samples of blood plasma were analysed of which 32 were samples were exposed to oxazepam and 10 were controls without exposure. Exposure times and treatment groups are shown in Appendix, table 3. The range of oxazepam concentration in blood plasma ranged from 3.85 to 31.4 ng/ml while all controls had no detection of oxazepam. The measured concentrations in water samples were lower than in the nominal concentration (mean= 7.16, S.D.=± 1.58). We confirmed that control tanks did not have oxazepam.

When I tested which predictors affected oxazepam concentration in the plasma, I found a significant relationship between AAS and oxazepam (n=32, linear mixed effects model, estimate =0.00074, S.E.= 0.00034, t = 2.16, p=0.045; table 2). Indicating that a higher AAS increases the concentration of oxazepam in blood plasma of brown trout (Figure 6). Additionally, the concentration of oxazepam was higher following a longer exposure period of seven days (estimate = 0.31, S.E.= 0.05, t = 6.77, p=0.015; figure 6; table 2). Although this did not reach statistical significance, I observed a trend between SMR and the concentration of oxazepam in blood plasma (estimate = -0.0032, S.E.= 0.0018, t=-1.75, p=0.091; table 2), suggesting that a higher SMR is associated with a decrease in oxazepam concentration in plasma. Furthermore, I observed a trend in the relationship between exhaustion time and the concentration of oxazepam which might show that a higher exhaustion time indicates an increase in the concentration of oxazepam (estimate = 0.0086, S.E.= 0.0047, t=1.829, p=0.079; table 2). Strain showed no effect on AAS (estimate = -0.061, S.E.= 0.044, t= -1.39, p= 0.18; table 2).

I observed a significant effect of MMR on the concentration of oxazepam (estimate=0.00074, S.E.= 0.00034, t=2.16, p=0.045, table 2, figure 7). Furthermore, I observed a significantly higher concentration of oxazepam during the longer exposure time of seven days (estimate= 0.305, S.E.= 0.045, t=6.77, p=0.015, table 2, figure 7). I found no statistically significant effect of exhaustion time, strain, and SMR on the oxazepam concentration.

Figure 6: Relationship between Absolute aerobic scope (AAS) and oxazepam plasma concentration (log10 transformed) of brown trout and the differences in concentration in blood plasma between exposure times of 168 hours (seven days) and 24 hours. The trend line represents a regression line, and the shaded area shows the 95 percent confidence interval for the regression line.

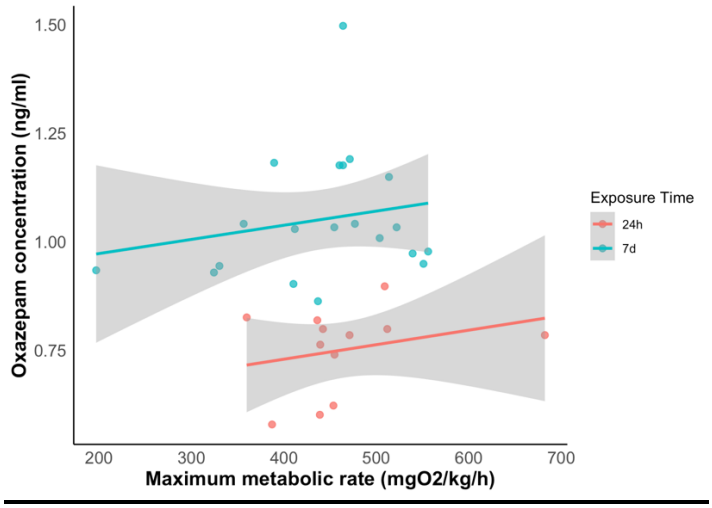

Figure 7. Relationship between maximum metabolic rate (MMR) and oxazepam concentration (log10 transformed) of brown trout and the differences in the blood plasma concentration between exposure times of 168 hours (seven days) and 24 hours. The trend line represents a regression line, and the shaded area shows the 95 percent confidence interval for the regression line.

Table 2. Results of the Linear Mixed Effects Models. Effect of predictor variables Absolute aerobic scope (AAS), exposure time seven days, and SMR on response variable oxazepam concentration. Effect of predictor Length and body score index measured in fat percentage on AAS, Standard metabolic rate (SMR), Maximum metabolic rate (MMR) and exhaustion time.

Response	Predictor	Estimate	Std. Error	t value	p value
Oxazepam concentration					
Model 1	AAS	0.00074	0.00034	2.16	0.045 *
	Exposure time 7 days	0.31	0.05	6.77	0.015 *
	SMR	-0.0032	0.0018	1.75	0.091.
	Exhaustion time	0.0086	0.0047	1.83	0.079.
	Strain	-0.061	0.044	-1.39	0.18
Model 2	MMR	0.00074	0.00034	2.16	0.045 *
	Exposure time 7 days	0.305	0.045	6.77	0.015 *
	SMR	-0.0039	0.13	-1.94	0.064
	Exhaustion time	0.0086	0.0047	1.83	0.079.
	Strain	-0.061	0.044	-1.39	0.176
AAS	Length	25.72	11.25	-2.29	0.032 *
	Strain	11.54	25.15	0.46	0.65

	Treatment	-15.02	26.60	-0.57	0.58
	Fat	-5.102	12.83	-0.39	0.69
SMR	Length	-7.29	1.59	-4.57	0.00013***
	Strain	0.76	3.56	0.21	0.83
	Treatment	-2.95	3.76	-0.79	0.44
	Fat	-3.33	1.82	-1.83	0.079.
MMR	Length	-33.28	11.99	-2.78	0.0107 *
	Strain	12.21	26.803	0.46	0.65
	Treatment	-19.47	28.38	-0.69	0.49
	Fat	-8.52	13.69	-0.62	0.54
Exhaustion	Length	-0.49	0.86	-0.57	0.58
	Strains	1.086	1.94	0.56	0.58
	Fat	0.404	0.98	0.41	0.69

3.2 Predictors of metabolic performance

In case of the respirometry trial 42 samples excluding the predictor variable fat in percentage and 33 samples including the variable fat percentage were analysed. Metabolic performance measured in AAS, SMR, and MMR ranged from: AAS = 166.8 to 604.2 (mean = 391.3, S.D. ±71.22), SMR 30 to 86.98 (mean = 51.39, S.D.± 13.701), MMR 196.8 to 682.1 (mean = 442.7, S.D.± 79.49). Exhaustion time ranged from 3.420 to 20 minutes (mean = 9.069, S.D.±4.71). Two fish were not exhausted or reached the 20 minutes threshold.

When I analysed the effect of strain, body condition measured in fat percentage, treatment, and length on AAS, I found that length has a significant negative association with AAS (estimate = -25.717, S.E.= 11.253, t = -2.285, p = 0.032, table 2), indicating that longer fish have lower AAS (Figure 8a).

Similarly, I found a very highly significant relationship between fish length and SMR (estimate = -7.2869, S.E. = -2.285, t = -4.569, p = 0.00013; table 2; figure 8b) indicating that longer fish have lower SMR values. Furthermore, I observed a significant effect of fish length on MMR (estimate = -33.279, S.E. = 11.987, t = -2.776, p = 0.0107; table 2; figure 8c) suggesting that longer fish have lower MMR values (Figure 8c). For the three analyses above, I found that none of the other predictor variables except length significantly affected AAS, SMR, or MMR (Table 2). There was no significant relationship between strain, treatment, weight, length and fat on exhaustion time (table 2, figure 8d).

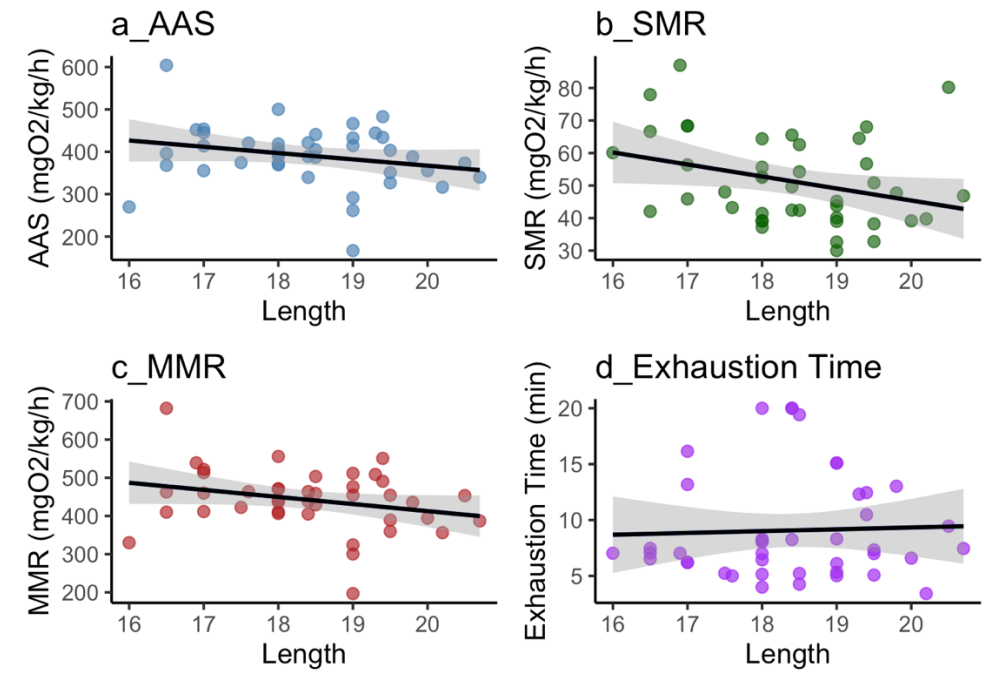

Figure 8. Statistically significant relationship between length (cm) of brown trout and absolute aerobic scope (AAS) (a), Standard Metabolic Rate (SMR)(b) and Maximum Metabolic Rate (MMR)(c). The relation between exhaustion time in minutes and length (d) is non-significant (Table2). The trend line shows the relation between AAS(a), SMR(b), MMR(c), exhaustion time(d) and length, and the shaded area shows the 95 percent confidence interval of the regression line.

There were no significant differences observed between AAS, SMR, MMR and exhaustion time (Table 2) between the two different strains from Norrfors and Skellefteå. Nevertheless, including two different strains in the data set provided a good variation of potential metabolic phenotypes, which is shown in Figure 9.

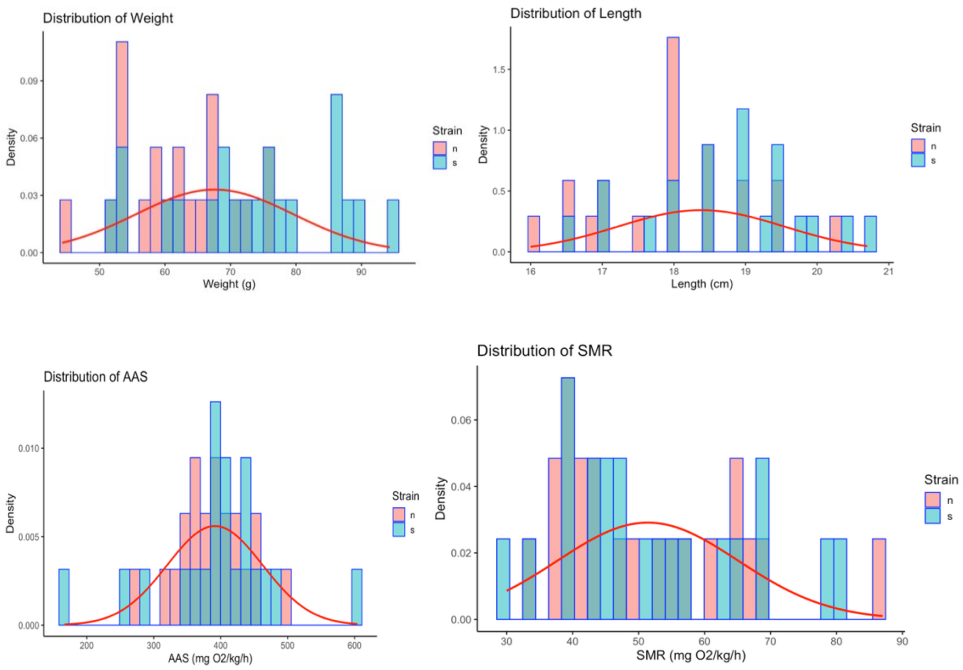

Figure 9. Data distribution of weight, length, AAS, SMR of Brown trout for the two different strains (Norrfors/Skellefteå) fitted with a normal distribution curve.

4. Discussion

Here, I tested whether various measures of metabolic performance predicted the bioconcentration of the pharmaceutical oxazepam in two different strains of brown trout over a short exposure of 24 hours and longer one of 168 hours (seven days). Additionally, I tested whether factors like body condition and size, strain, treatment group or time to exhaustion are predictors of metabolic phenotypes.

4.1 Pharmaceutical bioconcentration

First, I will discuss my prediction that absolute aerobic scope as a measure of metabolic performance, impacts the bioconcentration of oxazepam in plasma, resulting in a higher concentration with increased AAS. Literature on this topic how metabolic performance affects bioconcentration is very limited and a lot of variation exists regarding the study design. The study design differs in terms of use of different temperature regimes, testing of different pharmaceutical compounds individually, or in combination with other pharmaceuticals, and exposure of fish to different concentrations of pharmaceuticals and varying exposure times.

A possible explanation for the increase of oxazepam concentration along with a higher metabolic performance in my study could be because of its characteristics of hydrolytic instability and low water solubility, meaning it does not easily break down in water or by enzymatic hydrolysis (Han et al. 1977). Additionally, an increase in metabolic performance implies that fish will have increased gills movement (Millidine et al. 2008), which induces a higher blood flow in the organism and once oxazepam crossing the lipid layer of the gill surface into the blood stream by passive diffusion, oxazepam is distributed more rapidly. The delayed absorption of oxazepam, due to its lipophilicity (Dinis-Oliveira, 2017), may account for its sustained plasma concentration and slower excretion, despite increasing AAS levels.

Additionally, the increasing concentration of oxazepam in blood plasma along with a higher AAS, could be due to fish's specific physiology. Fish have only a single blood flow circuit, which is speeding up the uptake and distribution of water, oxygen and contaminants coming through gills, e.g. compared to mammals (Eddy & Handy 2012). In a study where trout were exposed to oxazepam, the results have shown that subordinates bioconcentrated more

pharmaceuticals than dominant individuals, along with the hypothesis that this might be due to higher ventilation and higher metabolic rates (McCallum et al. 2021). These results match with my findings that a higher gill movement induced by an in increase in AAS, implies an increase in oxazepam concentration. The hypothesis of the study by (McCallum et al. 2021) supports my results that higher AAS is related to increased concentration of oxazepam, emphasizing the importance of considering the link between and physiology, behaviour, pharmaceutical properties and environmental factors.

Another potential explanation is the high plasma binding potential of oxazepam (Chin et al. 2011), which is in line with findings by (Huerta et al. 2016; Cerveny et al. 2021a), that detected high levels of oxazepam in fish' blood plasma compared to other sampled tissues, like liver and muscle. The plasma protein binding potential describes the capacity to which a pharmaceutical can bind to a protein in blood plasma, making it pharmacologically inactive compared to its active free form. The binding potential determines on how much of the pharmaceutical is active and free to undergo the glucuronidation in the liver and following renal excretion (Bohnert & Gan 2013). Oxazepam has a high binding potential in fish (Henneberger et al. 2022), thus the slow release of oxazepam from its protein binding site, is a plausible explanation for its persistence in plasma along with a higher AAS.

Additionally, the findings in my study that SMR showed a slight negative trend, meaning it decreases with higher concentrations of oxazepam, could also be explained by oxazepam's protein binding potential. As SMR is only maintaining basic functions required in a resting condition (Metcalfe et al. 2016), it is a plausible explanation that the release of oxazepam in its free form is reduced during SMR. Another study on bioconcentration of synthetic estrogen EE2 in gold fish showed, similarly that estrogen was much slower depurated in fish than e.g. in mammals (Al-Ansari et al. 2013). This shows that this process of slow excretion and higher bioconcentration is not only limited to oxazepam or trout but can be find across fish species and various pharmaceuticals. These findings highlight the importance to investigate the mechanism of uptake and elimination of pharmaceuticals in fish in more detail.

When I looked at the relationship between oxazepam bioconcentration in plasma and the two exposure times, I found a significant increase during the exposure period of seven days compared to 24 hours. These results are in line with findings of previous studies which showed that oxazepam concentration in fish plasma increased during longer exposure periods (Heynen et al. 2016b; Cerveny et al. 2021a). Another study revealed an increase of oxazepam concentration in fish

plasma even during the dilution period, in which fish were not exposed to oxazepam (Cerveny et al. 2021a). A possible explanation for my finding that oxazepam bioconcentrations increased significantly more during longer exposure time, may be again oxazepam's high plasma protein binding potential and lyophilic character (Haddad 2018) which may lead to pharmaceutical persistence in plasma.

An alternative explanation could be, that a steady state, an equilibrium between uptake and excretion of a pharmaceutical, might have been reached only after 24 hours. In previous studies, steady state conditions were found to be drug specific and reached steady states at different timepoints (Sims et al. 2020; Burket et al. 2023). To assure that there was no peak after 24 hours, but a continuous increase I would need to investigate the plasma concentrations at additional time points.

Additionally, kidney and liver play a vital role in the pharmaceutical excretion potential in fish (Matthee et al. 2023). Enzymes called Uridine 5'-diphospho-glucuronosyltransferases (UGT) which induce the glucuronidation, meaning the metabolism of oxazepam to make it more water soluble for urinary elimination (Court et al. 2002) through renal excretion, might have an impact, too. This hepatic process in the liver and the detoxification through kidneys would need further investigation, to see if they are potentially involved in slowing down the process of elimination.

Nevertheless, the fact that oxazepam is increasing over longer exposure periods in fish plasma in few existing studies and its persistence in the in aquatic environment (Klaminder et al. 2015) is especially concerning. These findings show the importance of understanding how pharmaceuticals are bioconcentrated in fish. Environmental risk assessments and monitoring should include physiological diversity within fish populations and not only average exposure concentrations. Adding these biological variables help to account for when and where fish are more vulnerable when exposed to environmental stressors, for example during seasonal fluctuations, warmer temperatures and exposure to pollution.

4.2 Predictor of metabolic performance

In the second part, I will discuss what are possible predictors of metabolic performance to gain a clearer understanding of the factors that may indirectly affect bioconcentration.

Here, I tested whether factors like body condition measured in fat percentage and size, strain, treatment, group and time to exhaustion are predictors of metabolic rates. My findings revealed that length plays a significant role in predicting measures of metabolic performance. I found that length significantly affected metabolic performance, with increasing length showing lower metabolic performance (AAS), as well as lower metabolic rates in SMR and MMR. One possible explanation for my results that increased length of fish implies lower metabolic rates could be that the size of the gills limits how metabolic performance scales with body length. This means that bigger fish have a proportionally smaller gill surface, compared to smaller fish and thus oxygen consumption increases but metabolic rate decreases per unit mass, with increasing body length (Clarke & Johnston 1999). These findings are supported by previous studies which link length, size of gill surface and metabolic rates (Kuparinen et al. 2022).

My results showing that including predictor variables, might indirectly affect bioconcentration, is supported by findings from a recent study by (Heynen et al. 2016b) that showed an inverse relationship between weight and bioconcentration, with smaller perch concentrating more pharmaceutical after exposure. They hypothesized that the uptake of contaminants is decreasing with body size as gills are being smaller relative to body size. As length showed to be one of the main predictors of metabolic performance in my study, and metabolic performance showed to impact the uptake of oxazepam, I could suggest that length indirectly affects the bioconcentration of oxazepam. This could indicate an evolutionary advantage for larger fish bioconcentrating less pharmaceuticals than smaller fish. These findings could add value to aquaculture and breeding programs by emphasizing physiological traits that reduce bioconcentration risks and enhances ecological adaptability and survival.

Furthermore, my results showed that body condition measured in fat percentage, treatment and strain did not affect metabolic performance nor SMR, MMR or exhaustion time. I was expecting that different strains might influence metabolic performance, but a possible explanation might be that the two strains coming from river systems that are both in north of Sweden, located not too far from each other, have similar environmental and genetic selection pressures and hence do not show significant differences. There might be a better observable difference when using strains from geographical more distinct areas. Nevertheless, including different strains in this study still provided more variability of the metabolic phenotype data, as shown in the results. Additionally, it was important that treatment groups did not affect the metabolic phenotype, by showing that there

were no differences among the treatments in metabolic phenotype before being exposed in my study.

4.3 Conclusion

Pharmaceutical exposure is a rising environmental issue, yet we lack a clear understanding of what factors determine how much of a given pharmaceutical is bioconcentrated into fish tissues, as results have been variable to date.

By examining the effects of metabolic performance on bioconcentration I found that metabolic performance measured in AAS, and maximum metabolic rate had a significant effect on the bioconcentration of oxazepam in plasma of trout. Furthermore, the bioconcentration was significantly higher during the longer exposure time of seven days. Additionally, a relationship between body size in length and metabolic rates of SMR and MMR, and metabolic performance as AAS was observed. But no relation between all other predictors like body condition measured in fat percentage, strain, treatment, exhaustion time and metabolic rates, and no relation of any of the predictors on exhaustion time.

This study highlights the need for future research on investigating potential factors that determine the bioconcentration of pharmaceuticals in fish tissues and blood plasma over extended exposure times. In the next step, I suggest looking at additional factors that may influence bioconcentration and adding more complexity, as different temperature regimes, different species, different pharmaceutical compounds, and various age or developmental stages.

A better understanding of the mechanisms and factors driving bioconcentration of pharmaceuticals in fish including the role of metabolic performance is essential for predicting the movement of contaminants through aquatic food chains, evaluating long-term ecological risks, especially as pharmaceutical consumption continues to rise worldwide.

4.4 Limitations and future directions

There are several limitations associated with my study, and below I discuss them while also suggesting potential directions for future research. My study was performed in a laboratory. Laboratory conditions are necessary for experimental

control in a study like mine, but natural variabilities in environmental features like temperature fluctuations, changes in pH and presence of other pharmaceutical compounds could also alter both metabolic performance and pharmaceutical bioconcentration in a way I could not capture here. Furthermore, I suggest looking at the effects of long-term exposure, as I only used a short-term 24 hr or 7-day exposure. The effect of long-term exposure might reveal physiological adaptations to the pharmaceutical, and a potential to reach a "steady state" (where the rate of uptake and excretion stabilize), which are all important implications to understand the full potential of bioconcentration of oxazepam. To do this, I suggest sampling tissues samples at additional time points to detect if the increase continues or already reached a steady state at this point.

In this study, we sampled tissues from brain, muscle, liver and blood plasma, and in my thesis, I focused on plasma. Additionally, I propose to look at the oxazepam concentrations in kidney tissue as this where data from mammals show that detoxification and metabolism of drugs to some degree happens before the elimination through urine continues (Lin & Lu 1997). Further investigation into the other tissues collected will also reveal tissue-specific uptake patterns.

Finally, including different strains of trout that potentially come from more disparate regions in Sweden, or even across Europe may have allowed me to capture a wider variability in metabolic performance and metabolic phenotypes. Expanding these comparisons would also generalize my findings and make them more comparable with studies that are outside Sweden.

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Popular science summary

A fish is swimming through a cocktail of invisible pollutants that find their way into our ecosystem through various ways (see figure 10). Disposed medicine, excretion from animals and humans, pesticides and pharmaceutical used in agriculture and households, a variety of chemicals, all flushed or dumped and finding their way into rivers and lakes around the world.

What if I told you that the way fish swim and breath can affect how much of those pharmaceutical contaminants they take up, and eventually, end up on our dishes?

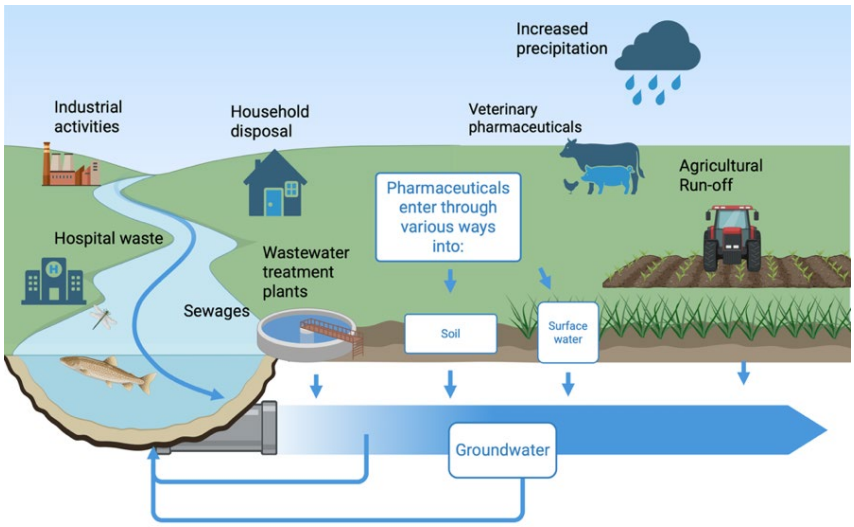

Figure 10. Figure shows the different origins of pharmaceutical contaminants and the ways they enter our ecosystem. Created in https://BioRender.com.

Pollution is quietly poisoning our aquatic ecosystems, including fish and many other aquatic animals. From antibiotics to painkillers to antidepressants, disposed pharmaceuticals are showing up in lakes, rivers and oceans, and fish are absorbing them. After absorbing those pollutants through skin and gills, some pharmaceuticals stay active and can have huge impacts at low dosages. Studies have linked oxazepam to boldness in fish and estrogen to feminisation in male fish, but we have not explored all the consequences yet.

A recent study by Aich and colleagues, 2023 examined the effect of fluoxetine, commonly known as Prozac, on guppies over multiple generations (three years).

The guppy's sperm vitality was permanently affected, which leads to reproductive loss and could have major consequences for the population. Very little is known about the mechanisms behind pharmaceutical concentrations in fish tissues, but obviously it affects the fish in our waters in various ways.

Another study on brown trout by (McCallum et al. 2021) revealed that fish in lower hierarchical positions would bioconcentrate more oxazepam, a commonly prescribed antidepressant, than fish in higher hierarchical positions. This might be linked to increased gill activity and higher metabolic rate. One explanation could be that fish in lower positions are more alert and stressed. If fish exposed to higher environmental pressures, show higher metabolic rates and this influences how much of pharmaceutical contaminants a fish absorbs, this would be important to consider for future studies, environmental risk assessments and monitoring.

I'm exploring how fish' metabolic performance, which means how fast they burn energy and how much they need for growth, reproduction, and coping with stress, affects how much pollution they take in. Will raising water temperatures and polluted environments have the consequence that fish shower higher metabolic rates are going to have higher concentrations of pharmaceutical in their tissues?

If we better understand how metabolism affects contaminant uptake, it helps to identify when and where fish are most vulnerable, and this can help us to protect freshwater and marine ecosystems, improve and support environmental regulations and conservation management strategies.

We are all in one boat on one water in the end!

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Appendix

Table 1. Output of linear mixed effects models for the best model and its response and predictor variable, and "Group" as random effect. Additionally, it includes the selection criteria R2, p-value, BIC and the Akaike Information Criterion (AIC). Predictor variables include strain (Norrfors/Skellefteå), exposure time (24/168 hours), Absolute Aerobic Scope (AAS), Standard Metabolic Rate (SMR), Maximum Metabolic Rate (MMR), exhaustion time.

Model	Response variable	Predictor variable	AIC	BIC
Exposure				
M1	**Oxazepam Concentration (log_oxazepam_2)**	**AAS, SMR, Exhaustion, Strain, Exposure time**	**-35.801**	**24.075**
M2		AAS, SMR, Exhaustion, Exposure time	-35.503	25.24
M3		AAS, SMR, Exposure time	-34.900	26.106
M4		AAS, SMR, Exposure time	-34.19	26.87
M5		SMR, MMR Exposure time, Exhaustion, Strain	-35.801	24.075
M6		MMR, Exposure time, Exhaustion	-33.37	24.570

Table 2. Output of linear mixed effects models for the best model and its response and predictor variable, and "Group" as random effect. Additionally, it includes the selection criteria R2, p-value, BIC and the Akaike Information Criterion (AIC). Predictor variables include strain (Norrfors/Skellefteå), treatment (control/exposed), fat percentage as body score index, length and weight.

Model	Response variable	Predictor variable	AIC	BIC	p
Respirometry					
M1	**Absolute Aerobic Scope (AAS)**	**Strain, Treatment, Fat, Length**	**384.99**	**395.46**	**0.02392***
M2		Treatment, Fat, Length	383.23	392.21	
M3		Strain, Fat, Length	383.28	392.26	
M4		Strain, Treatment, Fat	388.09	397.06	
M5	**Standard Metabolic Rate (SMR)**	**Strain, Treatment, Fat, Length**	**255.36**	**265.84**	**8.422e-05 *** **
M6		Treatment, Fat, Length	253.41	262.39	
M7		Strain, Fat, Length	253.98	265.84	

M8		Strain, Treatment, Fat	268.82	277.80	
M9	**Maximum Metabolic Rate (MMR)**	**Strain, Treatment, Fat, Length**	**389.67**	**400.14**	**0.0074****
M10		Treatment, Fat, Length	387.91	396.89	
M11		Strain, Fat, Length	388.11	397.09	
M12		Strain, Treatment, Fat	394.84	403.82	
M13	**Exhaustion**	Strain, Treatment, Fat, Weight, Length	214.56	226.53	
M14		Treatment, Length, Fat, Weight,	212.82	223.29	
M15		**Strain, Fat, Length**	**210.74**	**219.72**	

Table 3. All fish tested in respirometry divided in groups, strain (N/S), treatment (exposed/control), duration of exposure time (7 days/ 24 hours), number of fish tested (n). Every day one group was tested which included eight fish, four from each strain (N/S). They were divided into two treatment groups (exposed/control). Some individuals were excluded later due to technical issues with the oxygen detection.

Group	Strain	Treatment	Duration	n
1	N	Exposed	7 days	2
1	N	Control	7 days	2
1	S	Exposed	7 days	2
1	S	Control	7 days	2
2	N	Exposed	7 days	2
2	N	Control	7 days	2
2	S	Exposed	7 days	2
2	S	Control	7 days	1
3	N	Exposed	7 days	2
3	N	Control	7 days	2
3	S	Exposed	7 days	2
3	S	Control	7 days	2
4	N	Exposed	7 days	2
4	N	Control	7 days	2
4	S	Exposed	7 days	1
4	S	Control	7 days	1
5	N	Exposed	7 days	2
5	S	Control	7 days	1
5	S	Exposed	7 days	3
6	N	Exposed	7 days	4
6	S	Exposed	7 days	4
7	N	Exposed	24 hrs	4
7	S	Exposed	24 hrs	4
8	N	Exposed	24 hrs	4
8	S	Exposed	24 hrs	4

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