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Assessing the influence of upstream habitat conditions on benthic macroinvertebrate populations in tributaries of the Verdal River

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Acknowledgements

Going into this project I had very little knowledge of macroinvertebrates in general, let alone identification, life cycles, or how they are used as biological indicators. Over the course of the past year, I have gained an immense appreciation for the little guys and how they fit into riparian ecosystems.

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Abstract

Freshwater ecosystems are crucial for human civilization, providing essential ecosystem functions and services. With growing impacts of climate change and anthropogenic pressures, freshwater ecosystems are declining in their ability to maintain both ecosystem function and services around the world. As a result, once thriving biodiverse ecosystems are degrading at a rapid rate. Initiatives have been put in place across Europe to address these issues. Specifically in Norway, improving and maintaining river systems to be in good ecological standing has been a priority.

This study investigated the impact of upstream habitat conditions on ecological river health in small tributaries to the Verdal River in central Norway. Habitat conditions along rivers are not only influential on their local habitat but also downstream. Pollution and physical attributes, such as flow rates do have downstream effects. Research on upstream habitat impacts on local macroinvertebrates in Norway is limited. This study aimed to fill this gap by exploring the impact of upstream habitat conditions including average riparian conditions, stream gradient, and proportion of moving water on river health on benthic macroinvertebrates.

The following hypotheses were addressed: 1) The impact of upstream habitat decreases the further removed from the macroinvertebrate sample site they are, 2) Sites with more developed upstream riparian buffer zones will positively impact the diversity and water-quality indicator species composition, and 3) Upstream riparian buffer zones will have a larger impact than stream gradient or proportion of running water on downstream macroinvertebrate assemblages. Macroinvertebrates were collected via kick-sampling at the upper and lower end of each 31 stations along six tributaries. Local habitat characteristics were measured and followed the protocol of previous habitat measuring in the project area. Upstream habitat measurements were conducted separately and averaged over 50 and 100 meters stretches upstream of macroinvertebrate sample sites.

Results showed that none of the upstream habitat characteristics over either distance had any statistically significant impact on diversity or water-quality. For macroinvertebrate abundance, high scores for the riparian buffer over the 50-meter upstream stretch had a negative effect, resulting in a decrease in abundance. Local habitat variables were found to be relevant for both water quality and macroinvertebrate abundance. For water quality, the local environmental

gradient PC1 had a negative relationship with water quality. Along the same trend, both PC1 and PC2 local environmental gradients had a negative affect on macroinvertebrate abundance.

Introduction

Freshwater ecosystems are a vital component to the development and success of human civilization as they provide immeasurable ecosystem services such as drinking water, food, flood mitigation, climate regulation, as well as cultural and spiritual contributions to communities (Terje Bongard et al., 2018; Wang et al., 2023). However, with expanding human development and anthropogenically driven climate change, freshwater ecosystems' ability to provide ecosystem services, sustain ecological health, and withstand disturbances is negatively impacted (Mc Conigley et al., 2017; Wang et al., 2023). Globally, 65% of rivers are classified as under moderate to high threat due to human pressures with only 37% of rivers being free-flowing and largely unimpacted by human disturbance (Vörösmarty et al., 2010). As a result, ecosystems that were once rich in biodiversity, providing vital ecosystem services to humans are now degrading at a rapid rate impacting both ecosystem functions and human wellbeing. In response to this, many initiatives have gone into effect across the globe to combat these effects, including "The UN Decade on Ecosystem Restoration 2021-2030" which was brought forth on March 1st, 2019 (UN General Assembly, 2019). The aim of this decade is to have participating nations, including Norway, focus on restoring and ultimately halting the degradation of natural ecosystems.

Much of human impacts on river ecosystem biodiversity are driven by habitat loss and land use change, pollution, and invasive species (Mc Conigley et al., 2017; Petsch et al., 2021; Wang et al., 2023). As populations continue to grow, the demand to utilize land for agriculture and urbanization has increased dramatically in recent decades (Damanik-Ambarita et al., 2018; Krynak & Yates, 2018). Changes in land use for forestry, agriculture, and urban development impact both the chemical and physical characteristics of rivers, along with the structure and functioning of aquatic communities (Mc Conigley et al., 2017). In Norway, fish populations have been in decline in recent decades due to degrading habitat, putting a strain on the cultural and economic services they provide to the country (Liu et al., 2019). In this study's focus area in the Verdal region of central Norway, there has been a significant decline in brown trout populations compared to historic data (Eir Hol, 2018).

To address watercourse biodiversity loss and habitat degradation, Norway is following guidelines outlined in the European Union's Water Framework Directive (WFD) (Vannportalen, 2019). Under this framework, Norway is working towards improving all river systems to be in "good" ecological standing (Anonym, 2004). One tool utilized to improve and maintain freshwater ecosystems is riparian buffers. Riparian buffers are strips of vegetation along river and stream banks and serve as an ecotone between freshwater and agricultural zones (Cole et al., 2020). Benefits of riparian buffer zones include decreasing water temperatures (Le Gall et al., 2022; Mc Conigley et al., 2017), filtering chemical runoff (Cole et al., 2020), as well as contributing to aquatic food webs by way of plant debris and litter (Cummins et al., 1989; Le Gall et al., 2022). Currently, Norwegian law states that a minimal vegetative riparian buffer zone must be maintained unless there are structures that require direct access to the waterway (Vannressursloven 2000). Deciding the width of the riparian buffer zone is a responsibility given to local municipalities, of which Verdal municipality is passive in its application (Vilde Mürer, 2019) creating a lack of uniformity in riparian buffer zone application in the region. Considering the policies and guidelines that Norway is adhering to regarding restoring freshwater ecosystems, it is pertinent to study Norwegian river systems to evaluate the current ecological health and biodiversity status and improve restoration plans going forward.

Not only do riparian buffer zones impact the immediate stream area but also downstream as well. Dissolved oxygen (DO) levels, for example, decrease with increasing water temperature. Null et al. (2017) found that upstream DO levels do have a cascading downstream affect. Additionally, there have been studies looking at flow rates and their impact on macroinvertebrates downstream. In one study, it was found that reduced flow increased sedimentation and detritus fall out compared to higher flow of upstream reaches (Scholl et al., 2016). Increase in sedimentation and detritus provide benthic macroinvertebrates more hiding spots as well as food sources for grazer macroinvertebrates. As DO levels are critical for macroinvertebrate survival and riparian buffer zones help in regulating water temperature, there is relevance in investigating upstream riparian conditions and their relationship to downstream macroinvertebrate populations. As for flow rates, stream gradient and proportion of moving water are both associated components and are worth investigating to see if upstream measurements influence downstream macroinvertebrate populations.

To evaluate the ecological health of watercourses, meaningful data that reflects the state of the ecosystem must be collected. One such method is by utilizing biological indicator species. Biological indicator species are organisms whose biological response to their environment provide information regarding ongoing changes to an ecosystem (López-López & Sedeño-Díaz, 2015). Benthic macroinvertebrates are a commonly used biological indicator species group as they are diverse and abundant (Serrano Balderas et al., 2016) and are used in ecological quality measurements for WFD (van Puijenbroek et al., 2015). Species within benthic macroinvertebrates have varying responses to their habitat due to differences in their tolerances to pollutants, oxygen levels, as well as preferring different microhabitats and food sources (López-López & Sedeño-Díaz, 2015; Serrano Balderas et al., 2016). As a result, monitoring the diversity of macroinvertebrate assemblages is useful in investigating watercourse health as they provide predictable responses to environmental disturbances. Typically, benthic macroinvertebrates are used to evaluate local environmental conditions as their mobility in their ecosystem is limited (S. L. Johnson & Ringler, 2014; López-López & Sedeño-Díaz, 2015).

It is prudent to research the current health of river systems in Norway to meet the aims laid out in the Decade of Ecosystem Restoration and the European Union's Water Framework Directive, as well as inform future land management plans. While the local impacts of riparian buffer zones have been studied in many places around Norway, there is little research on impacts of upstream riparian buffer zones. Bridging this gap in knowledge will provide insight on the relevance of upstream riparian buffer zones along streams in relation to their ecological benefit. As such, this study investigates the impact of upstream riparian buffer zones along stream corridors on river health in small tributaries to the Verdal River in Norway.

Study aim

The Verdal river and its tributaries are in a region that is heavily impacted by agriculture with varying degrees of riparian buffer zones. Stretches of these tributaries have riparian buffer zones on both sides, one side, and no buffer zones at all. This study will investigate the impact of upstream riparian buffer zones on aquatic macroinvertebrate species. With this aim, the results from this study will serve as a steppingstone in the process of understanding the relationship between riparian buffer zones and benthic macroinvertebrate populations. This study is guided by the following research questions: How far upstream do riparian buffer zones impact benthic macroinvertebrates? What aspects of upstream habitat impact macroinvertebrates? Is there a relationship between more vegetated upstream habitats and local macroinvertebrate diversity and abundance?

Hypotheses

I. The impact of upstream habitat decreases the further removed from the macroinvertebrate sample site they are.

II. Sites with more developed upstream riparian buffer zones will positively impact the diversity and water-quality indicator species composition.

III. Upstream riparian buffer zones will have a larger impact than stream gradient or proportion of running water on downstream macroinvertebrate assemblages.

Methods

Study Site

The study site for this thesis was located along six tributaries of the Verdal River in the Trøndelag Fylke in Norway. The river begins close to the Swedish border and feeds into the Atlantic Ocean at the town of Verdal. The tributaries sampled include Brokskitbekken, Bjørkbekken, Follobekken, Korsådalsbekken, Rossvolbekken, and Skjørdalsbekken (Figure 1). The six tributaries identified in Figure 1 are located along a 12 kilometer stretch of the Verdal River and vary in length. Sampling stations were predetermined to keep in line with prior research conducted in this area. The number of stations on each of the selected six tributaries varied with 8 stations along Brokskitbekken, 5 along Bjørkbekken, 6 along Follobekken, 3 along Korsådalsbekken, 2 along Rossvolbekken, and 7 along Skjørdalsbekken.

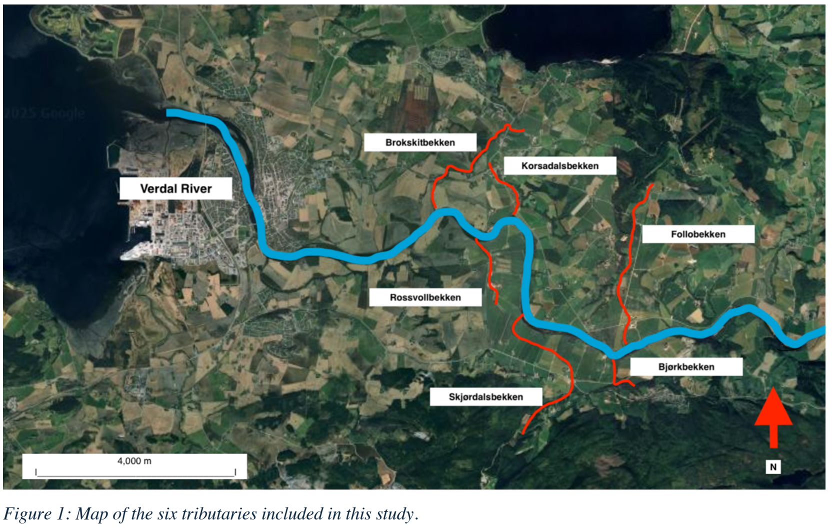

Figure 1: Map of the six tributaries included in this study.

The land surrounding the six tributaries is primarily agricultural, with the upper reaches of some of the tributaries residing in forested areas. Riparian vegetation varies between and within the tributaries with some stretches having vegetation on both sides, one side, on neither, or completely forested stretches. Korsådalsbekken runs through agricultural land plots with stretches that have riparian vegetation along the stream as well as open stretches and crosses one main road through a culvert. The stations further upstream run along the back side of neighborhood properties. Similarly, Brokskitbekken runs through agricultural land with three culverts along the stream. Stations 5, 6, and 7 of Brokskitbekken are adjacent to a local school that is highly trafficked by both people and cattle in the area. Bjørkbekken runs through a small, forested area close to urban development before going through a culvert and entering a forested area before meeting the Verdal River. Follobekken runs through agricultural land with sections of forested riparian vegetation buffers as well as open sections. The stream crosses three roads via culverts. At the downstream stations 1 and 2, just before joining with the Verdal River, Follobekken runs along a pig farm.. The two stations on Rossvolbekken have riparian vegetation along the streambanks and is surrounded by agricultural land. The upper stations on Skjørdalsbekken are in a forested area. Once the stream crosses Jamtlandsvegen the land surrounding the creek is agricultural. There are many culverts along this stream due to crossing Jamtlandsvegen as well as many driveways and truck roads before reaching Verdalselva. Riparian vegetation buffer zones are found along sections of the stream while other stretches are open.

Macroinvertebrate study species

Benthic macroinvertebrates are invertebrates that are visible to the naked eye. Body size metrics to qualify macroinvertebrates are defined as invertebrates with a minimum body length of 0.25 mm (Simon Pawley et al., 2011). As a diverse and well-studied group of insects, benthic macroinvertebrates are useful biological indicators for efficiently assessing water quality. Their diet is primarily composed of leaves, algae, and bacteria, and they are therefore dependent on conditions in the stream itself as well as energy inputs from the riparian vegetation for food. Additionally, benthic macroinvertebrates serve key ecological functions in riparian ecosystems and are one of the Biological Quality Elements that the Water Framework Directive utilizes to determine the ecological status of aquatic systems (Majaneva et al., 2024). This is done by collecting data on their diversity, abundance, and taxonomic composition (*Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy*, 2000). Many benthic macroinvertebrates orders were expected to be found based on previous research in the Verdal area, including Diptera, Oligochaeta, Ephemeroptera, Plecoptera, Tricoptera, Coleoptera, and Amphipoda (Louise Cathrine Rolstad Esdar, 2019; Hoberg, 2022).

Experimental Design

This study included both field work and laboratory work. Local vegetation and habitat characteristics were measured during fieldwork that took place from the end of August through the beginning of September of 2024. Local vegetation characteristics included the percentage of woody vegetation shade directly over the stream water, woody vegetation shade over the floodzone, as well as woody vegetation shade from the banks of the streams to the bankfull point. The current regulations regarding maintaining riparian vegetation buffer zones are limited, with the requirement being that a minimal vegetation buffer should be maintained (Vannressursloven, 2000). Upstream habitat measurements included gradient, fraction of running water, and riparian vegetation scores. Fraction of running water and riparian vegetation scores were measured in the field while gradient was taken from the public online database, Høydedata (KartVektet, n.d.). The upstream habitat measurements were conducted over the course of two different fieldwork sessions, one from the end of August to the beginning of September of 2024 and the second in November of 2024.

The amount of vegetation coverage is required to determine if there is a relationship between the percent of vegetation coverage and macroinvertebrate community composition and diversity on both a local and upstream scale. Foliage cover over and in the riparian zone along a stream is an important component as it directly impacts water temperature and subsequently the types of macroinvertebrates that are suitable for the area (Cummins et al., 1989; Le Gall et al., 2022). Other instream habitat characteristics that were measured included the number of woody debris and pools along the length of each station, percentage of stream sediment categorized by diameter size, stream depth and width, as well as moss and algae coverage. . Counting the number of woody debris in each station section is pertinent as they provide a food source for macroinvertebrates via biofilm development (Hrodey et al., 2008).

The explanatory variables of this study were the local habitat variables and upstream habitat variables.. Response variables included diversity, water quality, and abundance of the benthic macroinvertebrate assemblages. Duplicate sampling was conducted to validate data collection findings, resulting in two samples being taken from each station's start and end. In data analysis these duplicates were combined to reduce sample variability, account for sampling errors, and improve model assumptions. A total of 31 stations were sampled across six streams, totaling 119 samples that were taken in the field and analyzed in this study.

Sampling Plan

Kick-sampling

The aquatic macroinvertebrates sampling was done using the kick-sampling method (Letovsky et al., 2012) and is a typical method of measuring macroinvertebrate diversity (Majaneva et al., 2024). This sampling method involved placing a fine mesh kick-net on the riverbed and kicking the substrate directly upstream of the net vigorously. By doing this the macroinvertebrates in the sampling area were captured within the net. Both net size and time allotted for kicking up the substrate vary depending on the aims of the research (Hannah Hoberg, 2022; Letovsky et al., 2012; Louise Cathrine Rolstad Esdar, 2019; Majaneva et al., 2024). In previous master's thesis research on macroinvertebrates in tributaries of the Verdal River a 25 x 25 cm handheld net with a mesh size of 250 µm was used (Hannah Hoberg, 2022). Keeping in line with previous research in this area to maintain uniformity, the samples were taken using a 25 x 25 cm handheld net with a mesh size of 250 µm. The sampling duration in this study was sixty seconds of vigorous kicking split into two sets of 30 seconds covering a small area by the station location. Samples were taken within 1-2 meters of each station's start and end with a total of four samples per station.

After each 60 second kick sampling was completed, the bottom third of the net was held in the stream. At this time, large rocks and debris in the sample were cleaned in the running stream water flowing through the net. Once each large rock or debris was cleaned it was removed from the sample and returned to the stream. While some macroinvertebrates may have been lost in this process, it was done to keep the plastic bags used for containing and transporting the samples from breaking. Through this process, the samples were rinsed continuously for 5-8 minutes, leaving only smaller rocks, detritus, and substrate behind as well as macroinvertebrates. Once rinsing was completed, the sample was transferred to a plastic bag, taking care to thoroughly examine the inside of the net for any remaining macroinvertebrates. The plastic bag with the sample was then filled with a 96% ethanol solution and a label denoting the stream, station, subsample number, and date was added. To ensure the sample was not broken in transport, the bag with the sample was placed in a second plastic bag. Once the samples were successfully transported from Verdal to the lab at NMBU, they were stored in a freezer at 4 degrees Celsius to preserve the samples until identification.

Habitat measurements

Local habitat measurements were done along five transects in each station and included a range of variables (

Table 1). The transects were evenly spaced along each station according to station length. As macroinvertebrate sampling only took place at the end points of each station only transect 1, located at the downstream end point of the station, and transect 5, located at the upstream end point of the station, were included in this study. At each transect the width of the stream was measured. Along the stream width, the depth of the stream was measured at the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile markers. The average depth along the transect was used in this study. These markers were taken from left to right along the transect facing upstream. A square frame with an area of 1 m^2 was then placed along the transect. Placement of the frame along the transect was determined by a random number generator from 1-5. The result of the number generator determined which of the percentile markers used to measure stream depth the frame was to be centered around with 1 aligning the 10^{th} percentile and so on. Other measurements taken included the percentage of algae and moss coverage along the transect, substrate makeup, shade over the water, between the edge of the water and the bankfull mark, as well as the flood zone region. These percentages were determined by visual estimates. Substrate makeup was determined by estimating the percentage of the transect that was covered by substrate that had diameters of 0-2 mm, 2-20 mm, 20-100 mm, 100-250 mm, and over 250 mm. For this study, the average substrate diameter and average stream depth was used in data analysis. The number of pools and large woody debris (LWD) were counted as a total along the entirety of the station length. The sampling locations of benthic macroinvertebrates were at the start and end of the stations. As such, any pools and LWD that were not in the direct vicinity of sampling points did not impact macroinvertebrate samples. To take this into consideration, counts of pools and LWD were halved in the data. Samples from the start and end of a station were designated to have the same number of LWD and pools, which were half of the total found along the station length.

Table 1: Overview of local habitat variables and their measurement metric.

Variable	Measurement Metric
Station length	Distance (*m*) from the start and end points of the station. Measured in the stream.
Transect width	Width (*m*) of the stream at the transect.
Stream depth	Depth (*cm*) of water at the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile across the transect width.
Shadow over water	Percent (%) of shade directly over the stream by woody vegetation.
Shadow over stream bank	Percent (%) of shade directly over the stream bank by woody vegetation.
Shadow over the floodplain	Percent (%) of shade directly over the flood zone by woody vegetation.
Algae	Percent (%) of algae cover along the transect line.
Moss	Percent (%) of moss cover along the transect line.
Substrate	Percent (%) of the substrate with a diameter of 0-2 *mm*, 2-20 *mm*, 20-100 *mm*, 100-250 *mm*, and >250 *mm*.
Pools	Number of spots in the station that had a slow current and had a volume of 2 m^2 or larger.
Large Woody Debris (LWD)	Number of debris in the stream along the station length that were either >10 cm in length and >1 m in length or a collection of branches that collectively were of this size.

For this study, the upstream habitat measurements were the averages over the 50 and 100 meter stretches directly upstream of the sampling points of benthic macroinvertebrates. Gradient measurements were calculated by using topography information found on the public database, Kartvektet (KartVektet, n.d.). The fraction of running water was calculated by taking the length of the stream segment that was registered as a riffle or a rapid (moving water) by the total length of the stream segment. Riparian scores were calculated using the protocol laid out in *Stream Habitat Assessment Protocol for wadeable rivers and streams of New Zealand* (Jon Harding et al., 01/09) (

Table 2).

Table 2: Upstream riparian measurement score schematic. Scores range from 5-25 for each measurement.

Variable	Score 1	Score 2	Score 3	Score 4	Score 5
Vegetation composition (dominating)	Little/no vegetation	Non-woody vegetation	Trees<2 m in height	Trees 2-10 m in height	Trees>10 m in height
Riparian buffer width (m)	<1 m	1-5 m	5-15 m	15-30 m	>30 m
Surface shadows (%)	<10%	10-25%	25-30%	50-80%	>80%
Fraction of overhanging trees	<10% of station length	10-25% of station length	25-50% of station length	50-80% of station length	>80% of station length
Fraction of non-woody overhanging vegetation	<10% of station length	10-25% of station length	25-50% of station length	50-80% of station length	>80% of station length

Labwork

Benthic macroinvertebrate identification

Macroinvertebrate samples were identified in the lab to the lowest possible taxonomic level. Each sample was rinsed in the lab by placing the contents of the sample into a fine mesh sieve. Any remaining large rocks or debris were washed off in the sieve with water before being removed. After rinsing, the sample was placed in a petri dish with some distilled water. The macroinvertebrates were plucked out of the sample using tweezers and placed into another petri dish with a few milliliters of water. Each sample was methodically gone through twice to ensure that all the macroinvertebrates were removed. Macroinvertebrates were identified to the lowest possible taxon under a magnifying glass (with 4-40x magnification). In instances where more magnification was required, a Leica stereo microscope was used. This was typical for identifying the different species within the Plecoptera and Baetis genuses as differences between species were small. Ephemeroptera, Plecoptera, aquatic Coleoptera, and Gastropoda were all identified to species level unless the specimen was too immature or damaged. In cases of immaturity or degradation, specimens were classified to the lowest taxonomic level, either family or order. Diptera and Trichoptera were identified to family level. The following literature sources were used in identification: *Guide to British freshwater macroinvertebrates for biotic assessment* (Simon Pawley et al., 2011), *Stoneflies (Plecoptera) of Fennoscandia and Denmark* (A. Lillehammer, 1988), *Insektlære for fluefiskere* (Pål Krogvold & Ketil Sand, 2008), Våra snäckor (Bengt Hubendick, 1949), and *Trichoptera Larvae of Finland: A key to the caddis larvae of Finland and nearby countries* (A. Rinne & P. Wiberg-Larsen, 2017).

Data Analysis

Shannon-Weiner Index

To measure diversity, the Shannon-Weiner (SW) index was used as it considers both the species richness in the habitat as well as the relative species abundance. The resulting index output value describes the diversity of species within a community. The output value is positively correlated to species evenness and abundance. The higher the index value, the higher the diversity of species there is in the assemblage. The formula used to calculate the Shannon-Wiener index is as follows:

$$Shannon - Wiener\ index = \sum[\frac{number\ of\ individuals\ of\ one\ species}{total\ number\ individuals\ in\ the\ community}]$$

Average Score Per Taxon Index (ASPT)

The ASPT is a commonly used index in Europe that uses benthic macroinvertebrate metrics (Majaneva et al., 2024) and is utilized in Norway as a tool to measure effects of organic pollution and is also used as a general ecological health index (Veileder 02:2018 Klassifisering Av Miljøtilstand Eventuell Dato i Vann Økologisk Og Kjemisk Klassifiseringssystem for Kystvann, Grunnvann, Innsjøer Og Elver, 2018).

The ASPT index includes 85 taxa each with their assigned score ranging from 1-10. The score values for each taxon are reflective of their tolerance to organic pollution and eutrophication. A value of 1 is reflective of taxon with a high tolerance to pollutants and 10 is reflective of taxon with a low pollutant tolerance. The overall ASPT index score is an average of the tolerance scores of all the taxa species sampled. Calculating the ASPT score is as follows:

$$ASPT\ score = \frac{\sum taxa\ scores}{number\ of\ scoring\ taxa}$$

Statistical Analysis

The models tested remained the same across all analyses conducted to maintain uniformity. Model construction followed the same formula including at between one and two local and upstream habitat variables each. All statistical analyses were done in RStudio version 2024.12.1+563 (*RStudio: Integrated Development Environment for R*, 2024). Packages that were used included "vegan" for Shannon-Weiner diversity calculations and ordination, "dplyr" for organizing data, "MASS" for general linear modelling, "stats" for linear modelling, "ggally" for correlation plots of the habitat variables, "sf" for handling the upstream data, and "ggplot2" for plotting. The application, QGIS was used to combine spatial and elevation data (*QGIS Geographic Information System*, n.d.). See appendix 1 for guidelines used to pull together spatial, upstream habitat, and elevation data.

The chosen method of statistical analysis varied depending on the structure of the data as well as the type of response variable (continuous or count). To determine whether linear or unimodal ordination was the appropriate metho, a Decorana Correspondence Analysis (DCA) was done for both community composition and habitat variables. For axis lengths <3 linear models were appropriate whereas axis lengths >4 indicated unimodal (Šmilauer & Lepš, 2014). An unconstrained ordination method, Principal Component Analysis (PCA), was conducted on the selected local habitat variables to obtain environmental gradients (PC1, PC2, PC3) that drove variation in the dataset. These environmental gradients were later used in the models to encapsulate local habitat gradients for all analyses conducted. A Redundancy Analysis (RDA), a constrained ordination method, was used when looking at which environmental factors drove community composition.

Multiple linear regression analysis was utilized when analyzing the continuous response variables, SW and ASPT. Whereas for abundance, a count response type, a negative binomial regression was conducted. This method was selected to address the overdispersion present in the abundance count data. Finally, for model selection, the Akaike Information Criterion (AIC) was the chosen statistical method to evaluate the models for all analysis in this study. This method was chosen as it balanced the fit of the model while also accounting for complexity to avoid models that overfit the data. Models with the most support had lower AIC values explained the data of the models tested.

Results

Overall summary

The total macroinvertebrates collected across the 119 samples was 12,792. Of this total, several taxa stood out as large contributors of the total. Chironomidae made up 42.04 %, Oligochaeta 25.56%, and a distant third of *Baetis rhodani* at 5.63%. The percentage makeup of these macroinvertebrates varied greatly across the sites. At the ten sites with the highest abundance, Chironomidae, Oligochaeta, and *Baetis rhodani* accounted for 54.26%, 30.47%, and 1.72% respectively. On the other end of the spectrum, at ten sites with lowest abundance Chironomidae accounted for 15.86%, Oligochaeta 17.20%, and *Baetis rhodani* 15.32%. The station with the lowest number of benthic macroinvertebrates collected was at the upstream sampling point at station 1 on Bjørkebekken with only 12 specimens collected. The station with the most macroinvertebrates collected was the upstream sampling point at station 2 of Skjørdalsbekken with 1,781 macroinvertebrates. For summary of sites with highest and lowest abundances, including counts of Oligochaeta, Chironomidae, and *Baetis rhodani* see Appendix 2.

Assessment of Local Habitat Variation

The DCA on the benthic local habitat data resulted in an DCA1 axis length of 1.18 (Table 3). As such, the chosen analysis used for the analysis of local habitat data was a linear ordination (PCA).

Table 3: DCA of benthic macroinvertebrate composition. DCA1 explained 9% of the variation within the habitat variables while DCA2 explained 5.9%.

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.09	0.059	0.041	0.12
Additive Eigenvalues	0.09	0.043	0.046	0.012
Decorana values	0.09	0.053	0.021	0.0074
Axis lengths	1.18	0.96	1.34	0.53

The PCA showed that PC1 (39.9%), PC2 (15.91%), and PC3 (11.62%) accounted for a total of 67.44% of the variation in the macroinvertebrate count data. PC1 was strongly positively loaded by large woody debris, vegetative shade coverage directly over stream, along the banks, and in the flood zone. There was moderate positive loading from transect width and mean substrate. PC1 was strongly negatively loaded by mean stream depth of transect, algae presence, and number of pools. Only moss had relatively low loading on PC1. PC2 was strongly negatively correlated to mean substrate size and algae coverage. Vegetative shade along stream bank and moss had weak negative correlation. Both vegetation shading directly over the stream and vegetation shade coverage over the flood zone had very little correlation to PC2. Pools, large woody debris (LWD), and mean transect depth all had positive loading on PC2 to varying degrees. PC3 had strong negative loading from mean transect depth and moss coverage. On the other end of the spectrum, PC3 had strong positive loading to pools and algae. Moderate positive loadings on PC3 came from transect width and LWD. Weak negative loadings on PC3 came from mean substrate size, vegetation shading in the flood zone, along the banks of the stream, as well as shading over the stream.

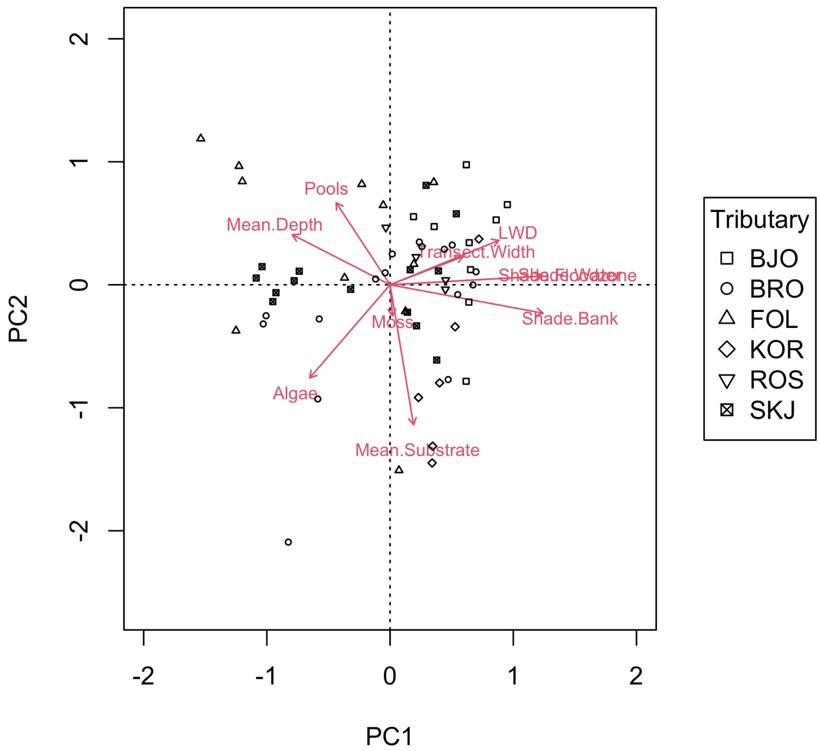

Figure 2: PC1 and PC2 accounted for 39.9% and 15.91% of the constrained variance respectively.

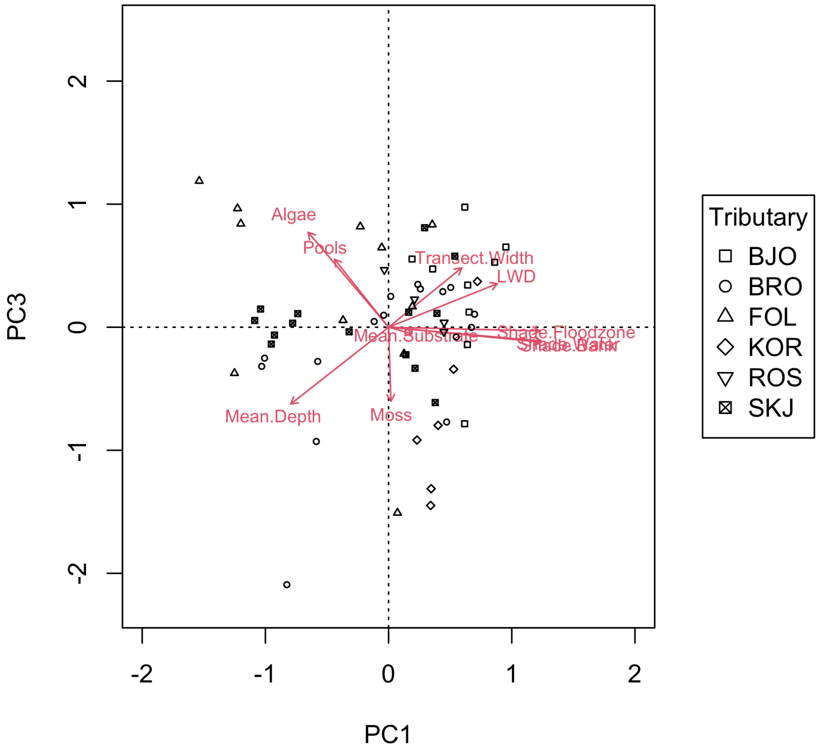

Figure 3: Biplot of PC1 and PC3. PC1 accounted for 39.9% of the variance while PC3 accounted for 11.62% of the constrained variance.

Shading from vegetation directly over the stream, along the bank, and over the flood zone were strongly corelated to one another along the PC1 axis (Figure 2 and Figure 3). Additionally, large woody debris and transect width also showed some correlation to one another along the PC1 axis (Figure 2 and Figure 3). Vegetation along the banks of the streams was inversely corelated to the mean depth of the water on the PC1, PC2 plot. While transect width and mean depth were inversely corelated along the PC1, PC3 plot.

Considering the information provided in the PCA analysis and the resulting plots, PC1 separated sites along a gradient where sites with positive PC1 scores were associated with more woody vegetation in the riparian zone along the stream. Additionally, sites with positive PC1 scores were associated with wider stream channels and more LWD in the stream. Sites with negative PC1 scores were more open with little to no woody vegetation in the riparian buffer zone with deeper and narrower stream sections. PC2 represented a gradient where sites with positive PC2 values were associated with more pools and finer substrate. Negative PC2 sites were associated with larger substrate material and more algae. The gradient along the PC3 axis ranged from sites with negative values that were deeper and mossier to sites with wider streams, more algae, large woody debris, and pools. As the aim of this study was to investigate the impacts of local and upstream variables on local benthic macroinvertebrates, PC1 was focused on in model selection as it encapsulated the most variance in the dataset.

Community Composition

A DCA on the benthic macroinvertebrate count data revealed an axis length of 2.39 (Table 4). As the axis length was less than 3, a linear ordination (RDA) was used.

Table 4: DCA of benthic macroinvertebrate composition. DCA1 accounted for 20.72% of the variation within the habitat variables while DCA2 explained 22.20%.

	DCA1	DCA2	DCA3	DCA4
Eigenvalues	0.2072	0.2220	0.1312	0.1185
Additive Eigenvalues	0.2072	0.2208	0.1312	0.1224
Decorana values	0.2842	0.2200	0.1495	0.1115
Axis lengths	2.3923	2.1845	1.7782	1.7281

In the constrained RDA model selection, twenty-six of the twenty-eight models had a ΔAIC < 2 indicating that nearly all the models had similar support in the data. Of the top fifteen model candidates based on AIC (

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Table 5), three of the top four models were all PC1 + fractRunning or avgRIPscore of either length of upstream stretch. Gradient variables were less supported as compared to fractRuning or avgRIPscore. PC2 and PC3 were not as supported as components to models as compared to models with just PC1 for local variables. As most of the models held similar explanatory power, with fifteen models with a ΔAIC < 1, adjusted R^2 was also looked at to determine the top model candidate (

Table 5).

Table 5: Overview of model candidates from the RDA analysis with a deltaAIC < 1, listed in order of Akaike most supported model and so on. one.. Adjusted ranked R^2 included to provide further insight.

Model	K	AIC	ΔAIC	Weight	LL	Adjusted R^2
PC1 + fractRunning100	3	189.34	0.00	0.06	-91.67	0.046
PC1 + PC2 + avgRIPscore100 + fractRunning100	5	189.47	0.13	0.05	-89.74	0.073
PC1 + PC2 + avgRIPscore100	4	189.55	0.20	0.05	-90.77	0.058
PC1 + PC2 + avgRIPscore50	4	189.56	0.22	0.05	-90.78	0.057
PC1 + fractRunning50	3	189.60	0.26	0.05	-91.80	0.042
PC1 + avgRIPscore50	3	189.80	0.46	0.05	-91.90	0.039
PC1 + avgRIPscore100	3	189.81	0.27	0.05	-91.90	0.039
PC1 + avgRIPscore100 + fractRunning100	4	189.85	0.51	0.04	-90.93	0.053
PC1 + gradient100	3	189.94	0.60	0.04	-91.97	0.036
PC1 + PC2 + avgRIPscore50 + fractRunning50	5	189.97	0.63	0.04	-89.98	0.065
PC1 + gradient50	3	190.12	0.78	0.04	-92.06	0.033
PC1 * avgRIPscore100 + gradient100	5	190.13	0.79	0.38	-90.06	0.063
PC1 + avgRIPscore50 + fractRunning50	4	190.17	0.83	0.37	-91.09	0.048
PC1 + PC2 + avgRIPscore100 + gradient100	5	190.18	0.84	0.37	-90.09	0.062
PC1 + gradient100 + fractRunning100	4	190.18	0.84	0.37	- 91.09	0.047

From

Table 5, the models with the largest adjusted $\boldsymbol{R^2}$ values were “PC1 + PC2 + avgRIPscore100 + fractRunning100” and “PC1 + PC2 + avgRIPscore50 + fractRunning50” with values of 0.073 and 0.065 respectively. As these two models were very similar to one another, variance partitioning was conducted to provide insight on whether the variables were improving the model or adding additional noise. From variance partitioning, it was found that avgRIPscore50 had an adjusted R^2 value of 0.048 indicating some explanatory power. Conversely, avgRIPscore100 had an adjusted R^2 value of 0.024, showing that it contributed less to the overall model than in the case of avgRIPscore50. In both models, the variables pertaining to the fraction of running water were found to have very low adjusted R^2 values, with fractRunning100 having an adjusted R^2 value of -0.0092 and fractRunning50 with an adjusted R^2 value of 0.0018. With such low adjusted R^2 values, the likelihood of the fraction of running water, regardless of distance, contributing unnecessary noise to the model was increased. As a result, the model chosen for macroinvertebrate assemblage variation was “PC1 + PC2 + avgRIPscore50” (Figure 4).

Effect test results for the selected model showed that the model was statistically significant with a p-value of 0.001 for explaining species assemblage (Table 6). The selected model explained 10.53% of the overall variance with RDA1 and RDA2 accounting for 70.04% and 24.17% of the constrained variance (Figure 4). Variance partitioning the selected model revealed that both PC1 and PC2 were statistically significant with p-values < 0.05, while avgRIPscore50 had a large p-value (Table 7). PC1 was the most significant with the lowest p-value at 0.004 and had the highest amount of variance at 1.20. PC2 followed with a p-value of 0.012 and a variance of 0.8987.

Table 6: Effect test (Anova) results for the selected RDA model.

	Df	Variance	F	Pr(>F)
Model	3	2.47	2.20	0.001
Residual	56	20.96		

Table 7: Anova results for the selected RDA model using variance partitioning.

	Df	Variance	F	Pr(>F)
PC1	1	1.20	3.21	0.004

PC2	1	0.90	2.41	0.012
avgRIPscore50	1	0.37	0.98	0.41
Residual	56	20.94		

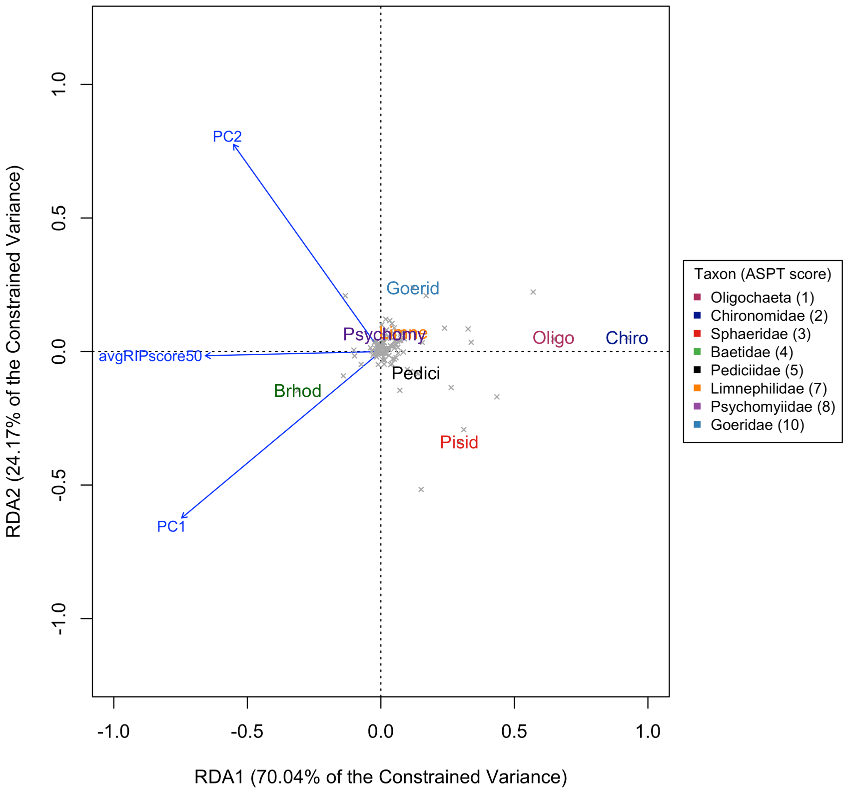

Figure 4: Biplot of the selected model, "PC1 + PC2 + avgRIPscore50." The eight species selected were the most abundant species in the data with unique ASPT scores. No taxon was collected with an ASPT score of 6 or 9 and are therefore not present.

The selected model showed that PC1, PC2, and avgRIPscore50 were all associated with negative RDA1 values. Along the RDA2 axis, PC1 was associated with negative values, PC2 with positive values, and avgRIPscore50 was closely aligned to zero. Oligochaeta and Chironomidae were strongly negatively associated with avgRIPscore50. Pediciidae was slightly negatively associated with PC2 while Pisidium was strongly negatively associated. Both Psychomyiidae and Limnephilidae were relatively neutral to all three variables in the selected model. Goeridae was moderately negatively associated with PC1. Only *Baetis rhodani* had a positive association with any of the variables in the model as it was moderately associated with both PC1 and avgRIPscore50. See Appendix 3 for full list of species found and shorthand names.

Macroinvertebrate Diversity

Diversity of macroinvertebrates found at each of the tributaries varied some, however, no one tributary was significantly different from any other (Figure 5). Rossvolbekken and Bjørkbekken had the highest average diversity values at 1.91 and 1.86 respectively. Korsedalsbekken, meanwhile, had the lowest average at 1.42. Bjørkebekken had the smallest range of SW values ranging from 0.894 to 2.32 while Skjørdalsbekken had the largest (0.376-2.43). For a summary of SW mean, min, max, and standard deviation see Appendix 4.

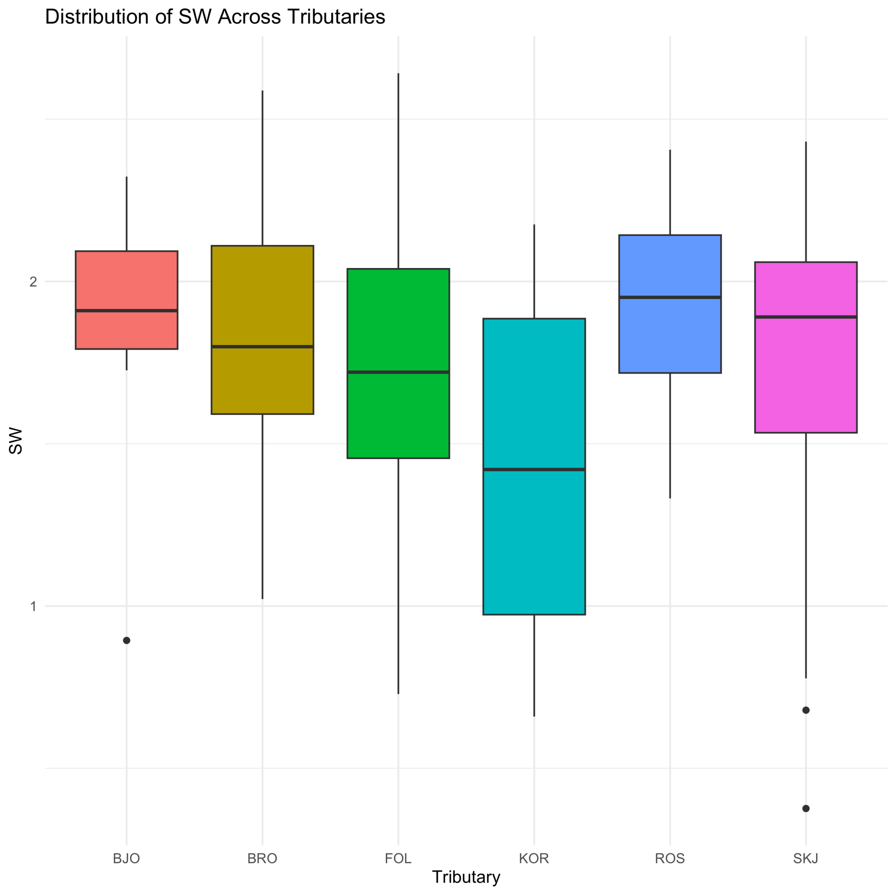

Figure 5: Boxplot distribution of SW diversity scores across the sampled tributaries.

A linear regression was chosen for modelling SW. Of the twenty-eight models run, only four resulted in ΔAIC values < 2 (Table 8). The model with the most AIC support at 15.1%was "PC1 + avgRIPscore50." Across all models with a ΔAIC < 2, upstream average riparian scores across either 50 or 100 meters were a variable in the models.

Table 8: Ten model candidates with the lowest AIC value for modelling SW. All values were rounded to the second decimal.

Model	K	AIC	ΔAIC	AIC Weight	LL
PC1 + avgRIPscore50	3	-76.96	0.00	0.15	41.48
PC1 + avgRIPscore100	3	-76.27	0.70	0.11	41.13
PC1 + avgRIPscore50 + gradient50	4	-74.99	1.97	0.06	41.50
PC1 + avgRIPscore50 + fractRunning50	4	-74.97	2.00	0.06	41.48
PC1 + PC2 + avgRIPscore50	4	-74.96	2.00	0.06	41.48
PC1 + fractRunning100	3	-74.96	2.01	0.06	40.48
PC1 + avgRIPscore100 + gradient100	4	-74.57	2.40	0.05	41.28
PC1 + gradient100	3	-74.53	2.43	0.05	40.27
PC1 + avgRIPscore100 + fractRunning100	4	-74.52	2.44	0.05	41.26
PC1 + fractRunning50	3	-74.47	2.50	0.04	40.23

Of the two models with a ΔAIC< 2, neither had statistical significance across any variables. In the selected model candidate, PC1 + avgRIPscore50, the p-value of avgRIPscore50 was lower at 0.12 compared to PC1 which had a p-value of 0.34 (Table 10). The estimates for each variable in the selected model regression summary (

Table 9) were positive meaning that as PC1 or avgRIPscore increase so does SW and can be seen in the prediction plot (Figure 6) derived from the selected model candidate. However, as neither PC1 or avgRIPscore50 had p-values < 0.05 from the regression summary, neither variable has a notable influence on SW. The R^2 value of the model was 0.02, further reinforcing that the model had low explanatory power.

Table 9: Regression summary table of the parameter estimates of the selected model, "PC1 + avgRIPscore50."

	Estimate	Std. Error	t-value	p-value
Intercept	1.15	0.39	2.99	0.004
PC1	0.04	0.11	0.40	0.69
avgRIPscore50	0.04	0.02	1.56	0.12

Table 10: Anova results for the selected model candidate with a response variable of Shannon-Weiner index number.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PC1	1	0.24	0.24	0.91	0.34
avgRIPscore50	1	0.64	0.64	2.43	0.12
Residuals	57	15.05	0.26		

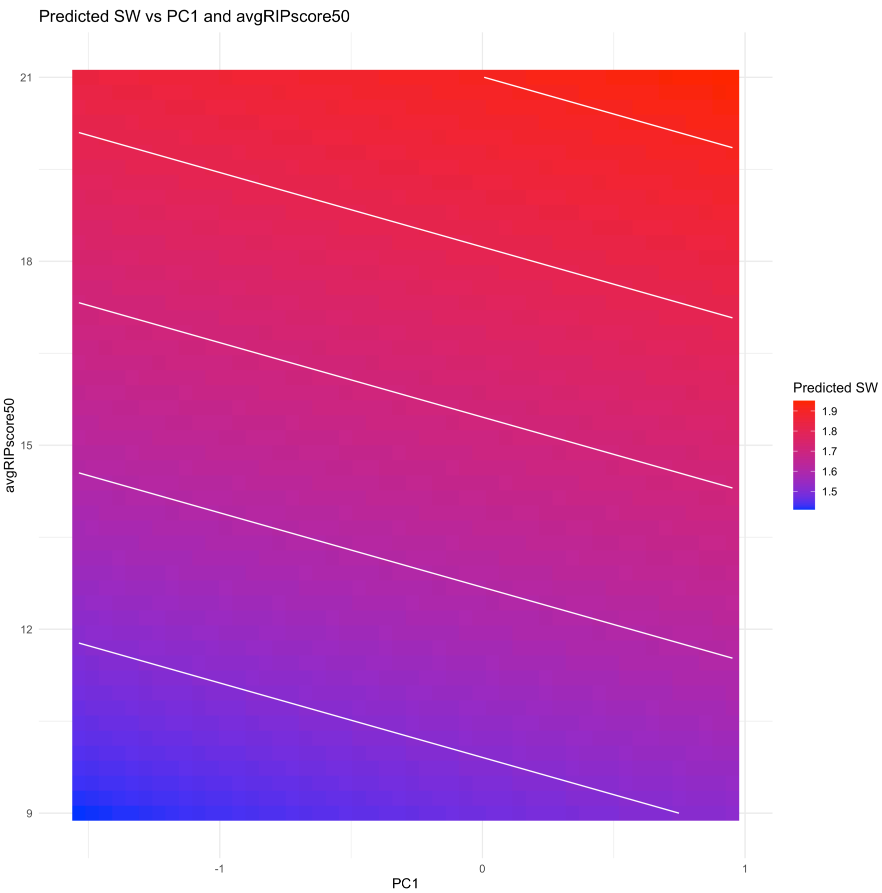

Figure 6: Plot with predictions derived from the selected model candidate, "PC1 + avgRIPscore50." Contour lines are denoted for every 0.1 increase in SW.

Water Quality (ASPT)

Water quality evaluated using ASPT ranged from 2.67 at the upstream sampling point of station 1 in Bjørkbekken up to 6.6 at the upper sampling point of station 4 on Brokskitbekken (Figure 7). Tributaries with the widest range of ASPT values were Brokskitbekken (4.14-6.6) and Follobekken (2.83-6.22). Korsedalsbekken had the smallest range of ASPT values (3.67-4.5) and the smallest standard deviation (0.336). Tributaries with large standard deviations were Bjørkbekken (0.966) and Follobekken (0.987). See Appendix 5 for summary of ASPT distribution for each tributary.

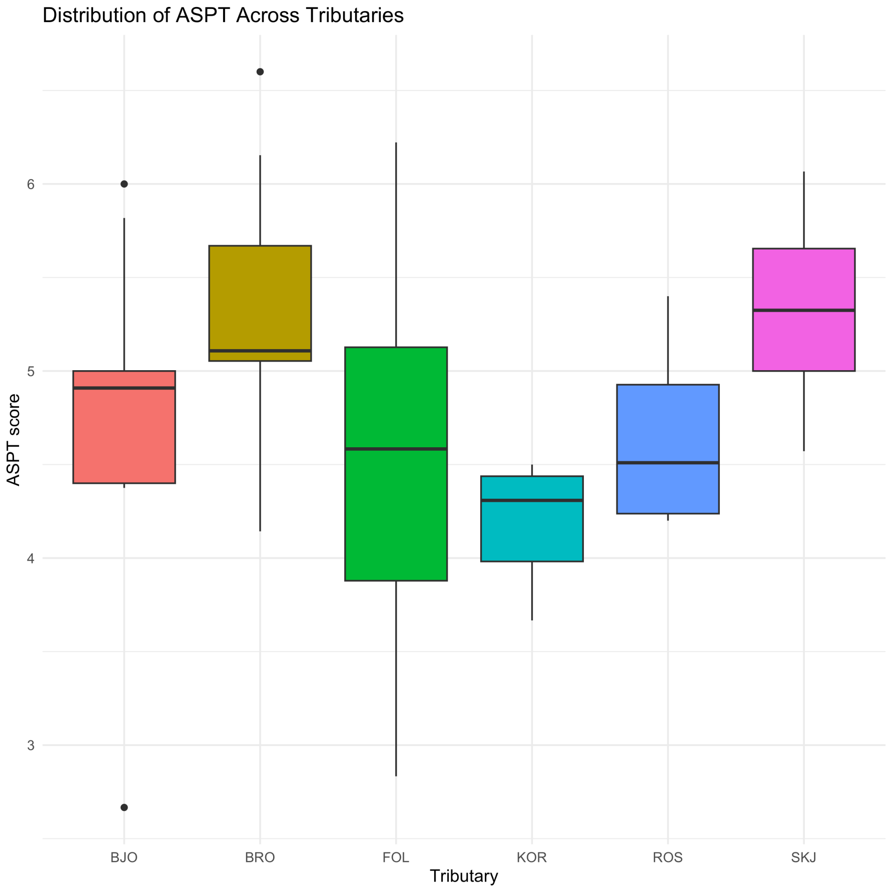

Figure 7: Boxplot for each of the six tributaries showing the range of ASPT scores at each station within each tributary.

Linear modelling was used to analyze the models for ASPT scores. The AIC analysis showed that 9 of the 28 models had a ΔAIC < 2, with the model candidate with the most AIC support (12.1%) as "PC1 + gradient100" and was the selected top model candidate for analysis (

Table 11). The top four models all included an upstream variable for gradient, with gradient 100 meters upstream represented in three of the four models. Following the top model, all subsequent models had AIC support of 7.4% or less.

Table 11: Overview of the ten model candidates with the lowest AIC values for modelling ASPT scores across the six tributaries. All values were rounded to the second decimal.

Model	K	AIC	ΔAIC	AIC Weight	Cumulative Weight	LL
PC1 + gradient100	3	-27.91	0.000	0.12	0.12	16.96
PC1 + gradient50	3	-26.97	0.95	0.08	0.20	16.49
PC1 * PC2 + avgRIPscore100 + gradient100	3	-26.81	1.10	0.07	0.27	19.41
PC1 + gradient100 + fractRunning100	3	-26.57	1.35	0.06	0.33	17.28
PC1 + fractRunning100	4	-26.47	1.44	0.06	0.39	16.24
PC1 + avgRIPscore100	4	-26.44	1.48	0.06	0.45	16.22
PC1 + avgRIPscore50	3	-26.19	1.72	0.05	0.50	16.10
PC1 + fractRunning50	3	-26.17	1.75	0.05	0.55	16.08
PC1 + avgRIPscore100 + gradient100	5	-26.00	1.91	0.05	0.60	17.00
PC1 * PC2 + avgRIPscore50 + gradient50	6	-25.87	2.05	0.04	0.64	18.93

The Anova analysis of the selected model showed no statistical significance for the upstream variable (Table 12). PC1 had the lowest p-value at 0.04while gradient100 had a p-value of 0.20. The sum of squares for PC1 and gradient100 were small compared to the sum of squares of residuals, showing that this model accounted for only 9.8% of ASPT variation, leaving much unexplained. In the summary regression (Table 13), PC1 had a p-value < 0.05 suggesting that PC1 impacts ASPT score. Additionally, PC1 had a negative coefficient suggesting that with increasing PC1 values leads to lower ASPT scores. On the other hand, gradient100 had high p-values both in the regression summary and in the Anova analysis. For predicting ASPT scores, values were derived from the selected model candidate and visualized how low PC1 scores along with steeper gradients 100 meters above macroinvertebrate sampling point tended to result in increases in ASPT scores (Figure 8).

Table 12: Anova analysis of the selected model, PC1 + gradient100. All values were rounded to the second decimal.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
PC1	1	2.69	2.68	4.50	0.04
Gradient100	1	1.02	1.02	1.71	0.20
Residuals	57	34.09	0.60		

Table 13: Regression summary of the selected model candidate. Values rounded to the second decimal.

	Estimate	Std. Error	t-value	p-value
Intercept	4.78	0.15	32.88	<2e-16
PC1	-0.36	0.16	-2.27	0.03
Gradient100	0.19	0.14	1.31	0.20

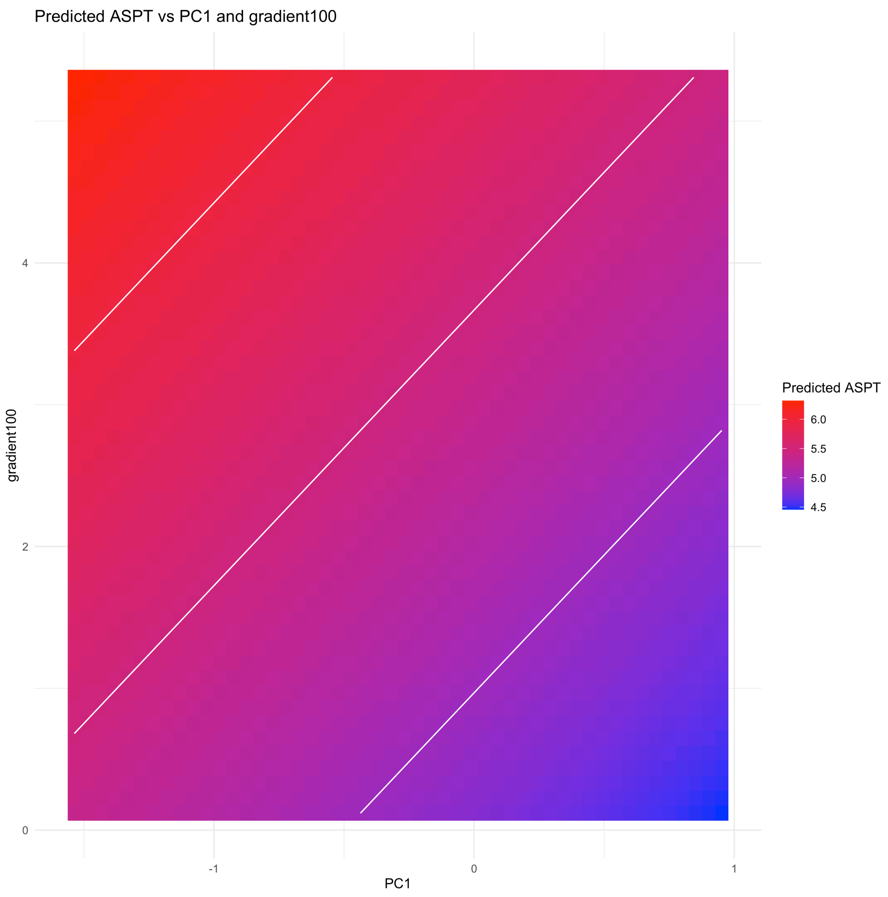

Figure 8: Prediction plot derived from the selected model, "PC1 + gradient100," for ASPT scores. Contour lines located at every 0.5 ASPT score.

Abundance of Macroinvertebrates

Abundance varied across the tributaries with some notable outliers (Figure 9). Korsdalsbekken had the highest average abundance across its samples with a mean of 415 macroinvertebrates. Bjørkbekken had the lowest average abundance with a mean of 72. Rossvolbekken had the smallest variance between stations with a standard deviation of 11.5, however, Rossvolbekken had the fewest number of samples of any tributary with only eight across the two stations. Skjørdalsbekken, Korsdalsbekken, and Brokskitbekken tributaries all had outliers of high abundance as well as disproportionate abundances of Oligochaeta and Chironomidae (

Table 14). See Appendix 6 for a full summary of abundance for each tributary.

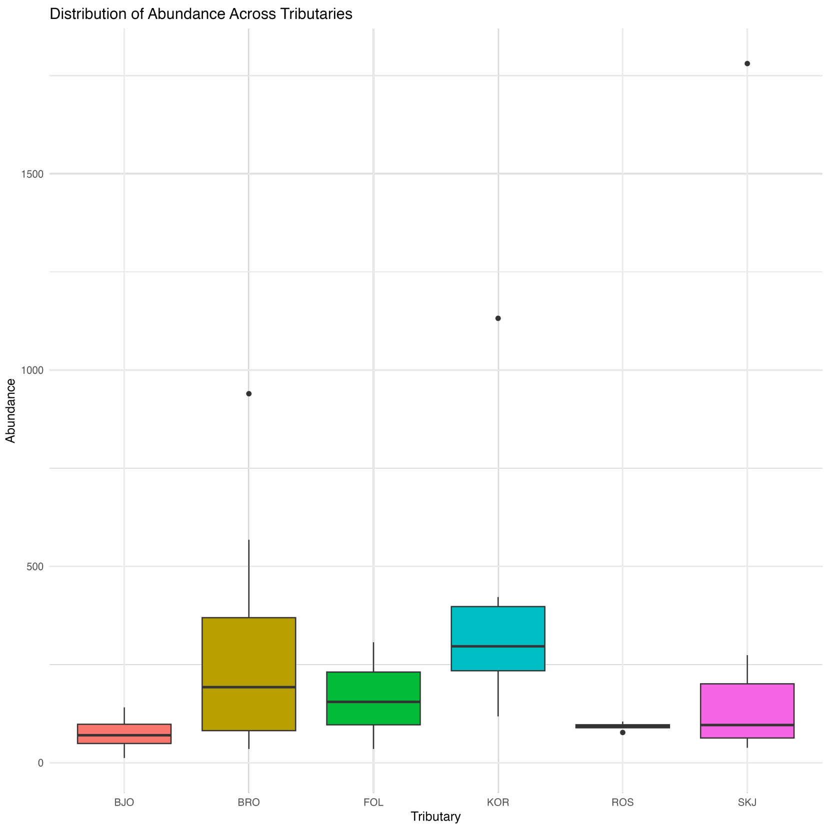

Figure 9: Boxplot of abundance distribution for each tributary. The boxes display the total abundance of all samples collected at each tributary.

Table 14: Overview of Oligochaeta, Chironomidae, and total abundance counts for each of the tributaries with high abundance outliers as well as counts across all samples for comparison.

	Oligochaeta	Chironomidae	Total Abundance	Percentage of total abundance
Korsdalsbekken	924	152	1132	95.05%
Brokskitbekken	212	608	940	87.72%
Skjørdalsbekken	18	1672	1781	94.89%
Across all samples	3269	5378	12792	67.60%

For modelling abundance, a GLM with a negative binomial distribution was used. In total, 6 of the 28 models had a ΔAIC < 2 (Table 15). The selected model with the most AIC support (18.7%) for abundance was "PC1 + PC2 + avgRIPscore50 + gradient50." The top three candidates all had PC1, PC2, and avgRIPscore50 variables in their models. The theta output of the top model was 1.696 (table x1) indicating that there was moderate overdispersion in the dataset, rendering the use of GLM with a negative binomial distribution appropriate.

Table 15: Ten models with the lowest AIC values in ascending order. All values rounded to the second decimal.

Model	K	AIC	ΔAIC	AIC Weight	LL
PC1 + PC2 + avgRIPscore50 + gradient50	5	746.13	0.00	0.19	-368.07
PC1 + PC2 + avgRIPscore50 + fractRunning50	5	746.28	0.15	0.17	-368.14
PC1 + PC2 + avgRIPscore50	4	746.33	0.20	0.17	-369.17
PC1 + PC2 + avgRIPscore100	4	747.93	1.80	0.08	-369.97
PC1 + PC2 + avgRIPscore100 + fractRunning100	5	747.95	1.81	0.08	-368.97
PC1 * PC2 + avgRIPscore50 + gradient50	6	748.02	1.89	0.07	-368.01

PC1 + PC2 + avgRIPscore100 + gradient100	5	748.14	2.00	0.07	-369.07
PC1 + avgRIPscore50 + fractRunning50	4	749.72	3.59	0.03	-370.86
PC1 * PC2 + avgRIPscore100 + gradient100	6	750.01	3.88	0.03	-369.00
PC1 * avgRIPscore50 + gradient50	5	750.43	4.30	0.022	-370.22

The residual deviance was lower than the null deviance suggesting that the predictors improve the model fit (Table 16). PC2 and avgRIPscore50 both had negative estimates and p-values < 0.05 in the summary of the binomial regression indicating that higher values of either of these values decreased the total abundance (

Table 17). PC1 had a borderline significant p-value in the binomial regression summary at 0.058 while gradient50 had a p-value > 0.05 suggesting that it did not strongly influence total abundance (

Table 17). From an Anova analysis, both PC1 and PC2 had p-values well below 0.05 and notable values for deviance explained at 12.37 and 13.28 respectively (
Table 18). As such, these variables were both statistically significant to the model and influence total abundance of macroinvertebrates. The average riparian score across the fifty-meter stretch above the sample location also had a p-value of less than 0.05 and was moderately significant on the abundance of macroinvertebrates. The gradient of the stream fifty meters upstream did not have a statistical significance. At lower PC1, PC2, and avgRIPscore50 values predicted abundance increased (Figure 10).

Table 16: Negative binomial regression summary of the model fit metrics of the selected candidate model, "PC1 + PC2 + avgRIPscore50 + gradient50."

Metric	**Value**
Dispersion Parameter (Theta)	1.696
Std. Error for Theta	0.288
Null Deviance	97.85 on 59 df
Residual Deviance	65.474 on 55 df

AIC	748.13
Log-Likelihood (2x LL)	-736.13
Fisher Scoring Iterations	1

Table 17: Negative binomial regression summary of the selected model candidate.

Predictor	Estimate	Std. Error	z-value	p-value
Intercept	6.93	0.61	11.36	<2e-16
PC1	-0.32	0.17	-1.90	0.058
PC2	-0.403	0.16	-2.55	0.01
avgRIPscore50	-0.093	0.035	-2.66	0.0079
Gradient50	-0.27	0.19	-1.47	0.14

Table 18: Anova analysis on the top selected model based on a chi-square test.

	Df	Deviance	Residual Df	Residual Deviance	Pr(>Chi)
Null			59	97.85	
PC1	1	12.37	58	85.11	0.00035
PC2	1	13.28	57	72.20	0.00033
avgRIPscore50	1	4.50	56	67.70	0.034
Gradient50	1	2.23	55	65.47	0.14

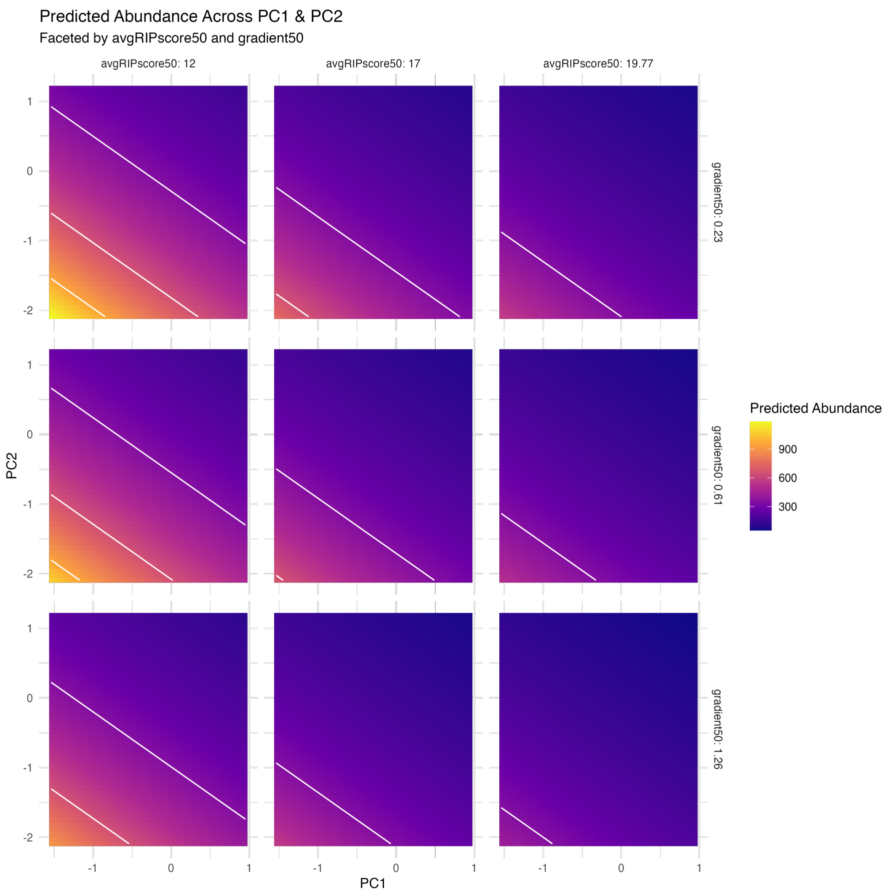

Figure 10: Prediction plot of the selected model candidate. Displays predicted abundance across PC1 and PC2 and along 10%, 50%, and 90% percentiles of both gradient50 and avgRIPscore50. Contour lines are denoted every 300 macroinvertebrate counts.

Discussion

The overall aim of this study was to explore whether, in addition to local habitat characteristics, upstream habitat characteristics such as average riparian score, stream gradient, and proportion of moving water had an impact on macroinvertebrates. Diving into the first hypothesis of this study, the impact of upstream habitat decreases the further removed from the macroinvertebrate sample site they are. Analyses for diversity, macroinvertebrate assemblage, and abundance favored models containing the average riparian score over the 50 meter stretch upstream of the sampling point over those that incorporated variables averaging across 100 meters upstream stretches. The only selected model candidate that included an upstream habitat variable across 100-meter upstream stretch was for ASPT with the gradient variable. Again, the effect test found it to not have a meaningful contribution to explaining ASPT results. Similarly, effect tests for diversity and macroinvertebrate assemblage found that upstream variables yielded no meaningful impact on local macroinvertebrates. As such, more developed upstream riparian zones did not positively impact diversity or water quality, as theorized in the second hypothesis of this study.

The selected model candidate for abundance did however indicate that the average riparian score over a 50-meters upstream stretch did contribute significantly to explaining macroinvertebrate counts. The results from this analysis indicate that upstream habitat conditions have impacts on some aspects of downstream macroinvertebrate populations. However, as the distance between the macroinvertebrate sampling sites and the upstream habitat increased the relationship weakened. Additionally, given that average upstream riparian vegetation score was a common component in the top selected model candidates across several analyses and was a significant contributor for modelling abundance, it suggests that the condition of the upstream riparian zone is a relevant component regarding macroinvertebrate populations, supporting the third hypothesis of this study.

Local habitat components were found to be statistically significant regarding macroinvertebrate assemblage and abundance. For ASPT, local habitat characteristics revealed tendencies but were not significant. These results are expected and in line with previous research as macroinvertebrates are typically used as bioindicators to provide insight on local habitat and water quality due to their limited mobility (Johnson and Ringler 2014; López-López and Sedeño-Díaz 2015).

Macroinvertebrate Diversity

Looking at the distribution of SW scores across the six tributaries, Bjørkebekken had the smallest range in SW as well as the smallest standard deviation suggesting that the tributary had more stable macroinvertebrate communities across the five stations sampled. Skjørdalsbekken, on the other hand, appeared to have more environmental stressors present, resulting in a large standard deviation and the wide range of SW values. Overall, the mean SW values for each tributary were close to the median values showing even distribution of diversity across the tributaries.

The selected top model candidate for modelling Shannon-Wiener diversity was a simple model of PC1 + avgRIPscore50. From the trends shown in the results section, as both the average riparian vegetation score 50 meters upstream and PC1 scores increased, predicted Shannon-Weiner scores also increased. As such it can be interpreted that diversity increases when both the local and upstream habitat has more woody vegetation. Under these environmental conditions, vegetation has been found to provide cooler water temperatures (Le Gall et al., 2022) and food sources for macroinvertebrates (Kumari & Sharma, 2018). However, the selected model candidate from this study yielded no variable that significantly contributed to explaining diversity variance and did not encompass much of what impacts diversity of macroinvertebrates in the six studied tributaries.

Factors that may be contributing to macroinvertebrate diversity that are not accounted for in this study include conductivity and nutrient loading. Increases in conductivity has been shown to decrease diversity, particularly of more sensitive taxa, as it can impact macroinvertebrates' osmoregulation (R. C. Johnson et al., 2013). Additionally, upticks in nutrient loading from sources, such as fertilizer and urban runoff as well as wastewater discharge (Carpenter et al., 1998). These inputs increase nutrients such as nitrogen and phosphorous which can lead to decreases in dissolved oxygen, negatively impacting macroinvertebrates (Carpenter et al., 1998). As the land directly adjacent to all six of the tributaries were dominated by agriculture, livestock, as well as urban housing it is possible that contaminants entering the streams impacted macroinvertebrate diversity to a higher degree than the components accounted for in this study. The establishment of riparian buffer zones have been used to mitigate impacts from nutrient runoff in to water systems (Aguiar et al., 2015). However, as neither established upstream riparian zones or well developed local vegetation were found to have a meaningful impact on diversity, nutrient loading cannot be ruled out as a potential component.

Another component to take into consideration when looking at drivers of macroinvertebrate diversity is emergence. The life cycles of macroinvertebrates are not all aligned in one time frame across a year. Seasonal changes in water chemistry, temperature, light, and hydrology are all factors that influence the timing of macroinvertebrate life cycles (Šporka et al., 2006). For example, Ephemeroptera, Plecoptera, and Trichoptera (EPT) abundances have been shown to be impacted by mean and maximum temperatures (Haidekker & Hering, 2008). With Plecoptera abundance typically decreasing in summer months when temperatures are higher whereas Trichoptera and Ephemeroptera increase (Šporka et al., 2006). This trend is in line with the abundance of EPT collected in this study where the total count for Plectoptera was 218 compared to 477 Trichoptera and 923 Ephemeroptera. As macroinvertebrate sampling was only done in one field session in late August through early September, the diversity results in this study are reflective of that snapshot in time.

Water Quality

Of the six tributaries included in this study, Follobekken and Brokskitbekken had the largest ranges of ASPT values suggesting that water quality conditions were much more site dependent. On the other end, Korsedalsbekken had the smallest range of ASPT scores showing that the water quality was more uniform across the stations in the tributary. However, it should be noted that Korsedalsbekken had only three stations and subsequently did not cover as much distance along the stream compared to other tributaries that had more stations.

Similar to the diversity analysis, the selected model for ASPT was simple with one local variable and one upstream variable, "PC1 + gradient100." PC1 was a contributing component to explaining ASPT and was shown to have a negative impact on ASPT score. From this, it may be interpreted that, at the local level in tributaries of the Verdal River, increased shade and more structurally complex sites tend to have lower ASPT scores. While there is a lot of research that shows riparian vegetation has positive impacts on macroinvertebrates in terms of various metrics (Cummins et al., 1989; Haidekker & Hering, 2008; Le Gall et al., 2022; Mc Conigley et al., 2017), there has been some evidence indicating that the size of stream is also a factor. One study found that small streams that had heavily vegetated riparian streams negatively impacted macroinvertebrates as opposed to larger streams due to food type availability (Ryan & Kelly-Quinn, 2016). As smaller streams are more likely to be entirely shaded as opposed to larger streams, autochthonous production is decreased subsequently decreasing food availability to the macroinvertebrate functional group of grazers. The small streams in this Ryan & Kelly-Quinn (2016) ranged in width from 3.5-4.0 m while the large streams had a stream width between 9.0 and 13 meters. Looking at the sites in the Verdal tributaries, all but two sites had stream widths that were less than four meters. Limitations in light to water surface may be a factor in the trend of decreasing ASPT score with increasing PC1 scores. However, further investigation would be required to explore this aspect.

The gradient of the stream 100 meters above macroinvertebrate sampling site had a positive coefficient, suggesting that steeper gradients might favor more sensitive taxa with higher ASPT scores. However, as seen in the Anova analysis, the p-value of gradient100 was insignificant, suggesting that this trend is weak and cannot be substantiated in this study. Of all the analyses done, ASPT was the only analysis where the most supported model whose upstream variable(s) did not include riparian vegetation scores of any upstream distance.

Overall, the r-square value of this model was low with only about 9.8% of the model explaining ASPT variation. So, while local habitat variables were shown to influence ASPT scores, much of what impacts ASPT was not included in this model. Two important aspects that impact ASPT that were not accounted for in this study were dissolved oxygen (DO) levels and temperature. DO and temperature have an inverse relationship as warmer water temperatures increase the rate of deoxygenation. As macroinvertebrates rely on oxygen, lower levels of DO negatively impact sensitive taxa while tolerant taxa increase (Berger et al., 2018). Additionally, there has been evidence that upstream levels of DO affect downstream levels (Null et al., 2017), furthering its relevance to the aims of this study.

Abundance of Macroinvertebrates

For modeling abundance of macroinvertebrates, the selected model candidate was, "PC1 + PC2 + avgRIPscore50 + gradient50." Both local variables, PC1 and PC2, had strong negative influences on the abundance of macroinvertebrates while avgRIPscore50 had moderate negative influence. Only the upstream gradient variable did not have a statistically significant p-value. With decreasing PC1, PC2, and upstream average riparian scores predicted abundance increased. Low PC1 scores in this study were associated with less structured habitats that had less woody riparian vegetation and LWD in the stream. Along similar lines,, avgRIPscore50 included components that summarized how well established the riparian zone was in terms of woody and non-woody vegetation coverage and width. Results from this study showed that increases in abundance aligned with lower avgRIPscore50 values, which were associated with narrower riparian zones that were more open and less shaded. This is line with other studies that have found that open riparian zones tend to have higher abundances, with some instances resulting in a 50% increase (Lester et al., 1994; McCORMICK & Harrison, 2011; Ryan & Kelly-Quinn, 2016). The presence of riparian vegetation is beneficial to stream systems in regards to water temperature and allochthonous input, however, its presence also impacts the light availability to the stream resulting in decreased primary productivity (Ryan & Kelly-Quinn, 2016). With a reduction in primary productivity macroinvertebrates that rely on autochthonous food sources, such as mayflies, may be negatively impacted (Ryan & Kelly-Quinn, 2016). Low PC2 values from this study are generally associated with sites that have coarser substrate and more algae presence. These conditions are favorable to macroinvertebrates, particularly grazers who rely on autochthonous production as well as some species of caddisflies that build their homes attached to the substrate in streams.

While looking at conditions that impact macroinvertebrate abundance it is pertinent to investigate what species increases or decreases as changes in abundance may not be uniform across all macroinvertebrate species. It is therefore imperative to examine what an increase in abundance implicates. In this study, the RDA analysis showed that both Oligochaeta and Chironomidae had negative associations with high riparian scores along the 50 meter stretch upstream from sampling. This was further solidified when looking at the ten sites with the highest abundance compared to the ten sites with the lowest abundance in this study. Where both Oligochaeta and Chironomidae made up a total of 84.73% of all macroinvertebrates collected in the ten sites with the highest abundance while only making up 33.06% of the total abundance across the ten sites with the lowest abundance. In other studies Chironomids have been found to increase in abundance and density at sites that are not shaded, particularly if they are small streams (Kiffney et al., 2004; Ryan & Kelly-Quinn, 2016).

The implication that species like Chironomidae and Oligochaeta are largely responsible for increases in abundance under low PC1, PC2, and upstream vegetation conditions is further fortified when examining what the absence of riparian vegetation implicates. It is well documented that a benefit of riparian vegetation is the reduction in pollutant runoff in to stream systems (Aguiar et al., 2015; Anbumozhi et al., 2005). In areas where land is used for agriculture, such as in the case of this study, high levels of nitrogen and phosphorous, are common as they are found in fertilizers that are used. The result of overloading river systems with these components can lead to eutrophication and "dead zones" where there is little to no dissolved oxygen in the water available to aquatic organisms including macroinvertebrates. Implementation of buffer zones have been found to not only reduce the amount of pollutants entering the water system but consequently increase the amount of dissolved oxygen (Aguiar et al., 2015; Anbumozhi et al., 2005). Under these conditions, species with higher tolerances, such as Chironomidae and Oligochaeta, would be able to withstand higher levels of pollution compared to more sensitive taxa.

Diving further into what types of vegetation are most effective, Aguiar et al. (2015), found that buffer zones of different vegetation types did reduce pesticide runoff with large woody vegetation with trees being best followed by shrubs and grass. Taking this into consideration, this study found both local woody vegetation coverage and upstream woody vegetation coverage were inversely related to abundance, it suggests that macroinvertebrate species with higher pollutant tolerances can dominate open areas. As average upstream riparian scores were found to be meaningful in terms of macroinvertebrate abundance, if to a lesser degree than local vegetation, it suggests that pollutant runoff entering a stream at higher concentrations upstream may still have downstream effects felt by macroinvertebrates. Both Chironomidae and Oligochaeta have low ASPT scores (1 and 2 respectively) and have higher tolerances to pollutants relative to other ASPT scoring taxon and as such are more likely able to survive and take over sections of streams that have less buffer against pollutant runoff.

Limitations in the study

This study had several limitations that need to be considered when looking at the results. Local habitat measurements were taken by four different people. As several aspects of local habitat measurements were determined by visually estimating observations, variation in the measurements is to be expected. Given the time and logistical constraints involved in the fieldwork it was not possible to have one person do all the habitat measurements. Macroinvertebrate sampling would have ideally been conducted multiple times throughout the year to capture a more accurate picture of abundances and species found in the tributaries (Šporka et al., 2006). By sampling at different times of the year the strength the findings in this study would, that local and upstream habitat variables are not strong influences on macroinvertebrate diversity. Again, due to time constraints of the project, multiple sampling trips to Verdal were not within the scope of this project. Sampling took place from August to September 9th of 2024 so weather and precipitation changes may have affected the samples collected. Macroinvertebrates may have also been lost when transferring samples from the net to the baggies used for storage as well as when samples were rinsed of debris both in the field and in the lab.

Regarding identification of the benthic macroinvertebrates, errors may have occurred due to inexperience. However, this is more likely to have occurred on the species level than family level. When identification could not be determined confidently down to species level, the macroinvertebrate were identified down to order or family though mistakes may have still occurred. Incorrect identification in this study would impact the results covering diversity, ASPT scores, and macroinvertebrate assemblage but would not have affected analysis on abundance. Finally, in the lab samples were split into subsamples, if a species count went over

40 in a subsample, that species total was multiplied by how many subsamples the sample was divided into. The only taxon this happened with was Chironomidae and Oligochaeta. As a result, in these instances the totals were estimations for 10 of the 119 samples and are denoted in Appendix 7.

Further Research

The results found that average riparian condition in the 50 meters upstream section was a significant upstream habitat characteristic when looking at macroinvertebrate abundance. However, when the upstream section extended to 100 meters this relationship did not hold. As this study only had two upstream section lengths, it would be pertinent to look at more frequent distance intervals to see how far upstream does the riparian conditions impact abundance. This would give a better idea as to how far the relationship between riparian conditions and macroinvertebrate abundance is. In the same vein, while diversity and ASPT showed no significant relationship with any of the upstream habitat characteristics included in this study these results are only for habitat measurements spanning a minimum of 50 meters upstream and cannot speak for stretches less than 50 meters.

While this study included riparian habitat measurements for both local and upstream variables, they were not entirely uniform. The upstream riparian score was the average of five components over either a 50- or 100-meter stretch. Local habitat variables, on the other hand, were gradients that encapsulated 10 measured characteristics. To improve continuity between local and upstream variables it would be prudent to have them both measure the same metrics. This could be done by either measuring local gradient, riparian condition score, and proportion of running water, or measuring the 10 local characteristics at designated distances upstream of macroinvertebrate sampling. Finally, as diversity is not uniform over the course of a calendar year due to differences in emergence, sampling at different times of the year would strengthen results regarding how local and upstream habitat variables influence macroinvertebrate diversity.

Conclusions

This study explored what, if any, upstream habitat conditions impacted macroinvertebrate populations downstream. To investigate this topic three hypotheses were investigated; I. the impact of upstream habitat decreases the further removed from the macroinvertebrate sample site they are, II. sites with more established upstream riparian conditions will positively impact the diversity and ASPT metrics of macroinvertebrates, and III. upstream riparian conditions will have a larger impact than stream gradient or proportion of running water on downstream macroinvertebrate assemblages.

The first hypothesis of this study was found to be partially supported. For both water quality and diversity no upstream variable played any significant role over either 50 or 100 meters. In the case of macroinvertebrate abundance, the average riparian score across the 50-meter stretch above the sampling site was found to be a contributing component for downstream abundance. This relationship, however, did not extend to the average riparian scores over the 100-meter stretch above sampling. A takeaway from this is that while benthic macroinvertebrates are generally used as biological indicators for local habitat influences outside of the immediate local area are still at play. For restoration and management plans this reinforces the concept of connectivity between and among natural systems and that successful ecological management extends beyond the immediate focus area.

The second hypothesis was not supported by the findings of this study as neither water quality or diversity were positively or negatively impacted by the average upstream riparian conditions. While upstream riparian conditions were not significant influencers on water quality, the local habitat gradient, PC1, was. Low PC1 values, i.e. habitats that were more open and less complex, were indicative of increased ASPT scores. While initially this may seem counter intuitive, it is important to keep in mind the parameters of this study. All the tributaries studied were relatively small, increasing the likelihood of light becoming a limiting factor at sites with high PC1 values. This would in turn, create habitat that is not suitable for benthic macroinvertebrate taxa that rely on macrophytes for food or shelter. As a participating party in the Water Framework Directive, Norway strives to bring all its rivers to good ecological standing and maintain them as such. Given that a component to measuring ecological quality of rivers is benthic macroinvertebrates, and by extension, ASPT scores, plans for restoration and management must account them. In the case of small tributaries like in this study, it may be worthwhile to adjust riparian buffer zone qualities such as width and density, to facilitate habitats that allow for the most varied habitat.

The third hypothesis was partially supported as the only upstream variable that showed any impact was average riparian zone conditions along the first 50 meters above sampling. At first glance, this may be contributed to the fact that more established riparian buffer zones upstream contribute to regulating water temperatures, provide food for grazer macroinvertebrates, and increasing filtration of pollutant runoff. All these components are favorable for a wide range of macroinvertebrates, including more sensitive taxa like EPT. However, the results from this study showed that abundance increased with less established upstream riparian zones. This, in turn may be due to less-than-ideal habitat conditions, creating an environment in which sensitive taxa struggle and more tolerant taxa can dominate. When approaching management and restoration of river systems, which is an objective that Norway has identified, it may not be in the best interest of overarching management goals to create conditions such as those found in the Verdal tributaries with high macroinvertebrate abundance. As benthic macroinvertebrates are both a vital component to the aquatic food web and are biological indicators for ecological health, it is a balancing act in terms of management. On one hand high abundances mean more food for higher trophic levels such as fish, which are a crucial component to the culture and economy of the Verdal area. On the other hand, if water conditions are such that populations are largely dominated by tolerant taxa, it may be indicative of unsuitable habitat for brown trout or salmon.

As Norway works towards addressing fish population instability and bringing all rivers and streams up to good ecological condition the components that contribute to diversity, water quality, and abundance of benthic macroinvertebrates need to be taken in to consideration. This study provided beginning steps of understanding the relationship between upstream habitat conditions and downstream benthic macroinvertebrates. However, further research is needed to better understand the dynamics at play and best inform management plans going forward.

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Appendix

Appendix 1 – Routines for transforming field data on riparian zone variables

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Routines for transforming field data on riparian zone variables, mesohabitats and elevation to 1-meter resolution spatial data frame along tributary lines in R – also including estimation of upstream attributes

Background

In this document I will describe how to proceed from registering tributary (meso)habitat and riparian zone data using cell phone apps to construct points with one meter resolution along the tributaries where all one-meter points will have attributes consisting of all measured variables and elevation along with multiple upstream variables extending from 50 meters to 200 meters. This one-meter resolution data will be formatted as a spatial data frame that can be easily merged into other spatial data frames, such as for instance benthic invertebrate sampling points and fish-density sampling locations, simply by using the sf::st_nearest_feature() in R.

Registration of data

Data on mesohabitats and riparian zone attributes get registered as georeferenced waypoints using either google forms (riparian zone variables, **Figure 1**) or Qfield-forms (mesohabitats, **Figure 2**). When using google forms a GPS or cell phone GPS-app must be used simultaneously to register waypoints with matching point ID as the google form does not have a built-in georeferencing system.

The registration routine is the same when using both apps. All registrations start at the tributary mouth and when entering a new mesohabitat (either pool, riffle or rapid) area or a new riparian zone type (defined as in Harding et al. (2009)) a waypoint is marked in the app with choice of mesohabitat type and/or the five riparian variable scores (i.e., Dominating vegetation composition, Riparian zone width, Surface canopy shading, Fraction overhanging trees, Fraction of non-woody overhanging vegetation, -each scored between 1 and 5).

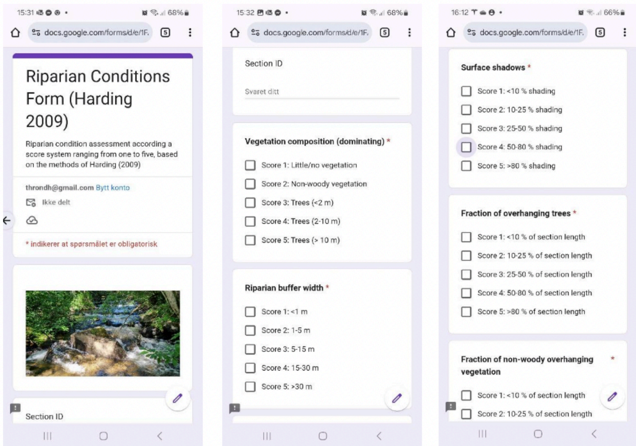

Figure 1. *Screen dump of the first part of the google form application used for registering five riparian zone variables. The data gets stored in web-cloud instantly and by entering a section ID (three-letter tributary abbreviation and running number) corresponding to same waypoint ID in the Norgeskart Friluftsliv app (https://ture.no/), matching riparian zone attributes can be joined with spatial coordinates later.*

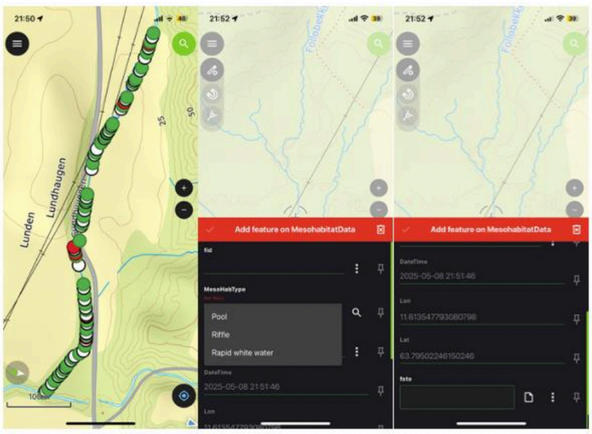

Figure 2. *Screen dump of Qfield-app in operation for registering mesohabitats. Figure was provided by Ingeborg Aasebø.*

An example of data entries of riparian zone variable scores for the Korsådalsbekken tributary are shown in **Figure 3** and for mesohabitat assignments in **Figure 4** (left panel).

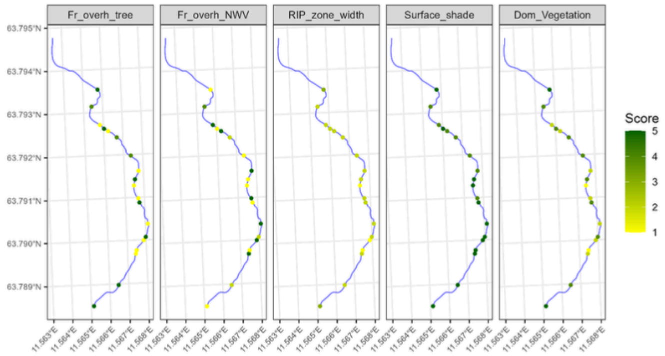

Figure 3*. Raw data of riparian zone variable scores in Korsådalsbekken during fall 2024, based on inputs using the google form app.*

Steps from raw data to construction of upstream habitat data

After downloading data and reading them into QGIS and R (using sf::st_read()), we must undertake a couple of steps to get to desired spatial data frame with one-meter resolution points.

1. Align assignment points to tributary line
2. Use assignment points to split the tributary line into habitat-specific segments
3. Establish one-meter resolution sampling points (1mSP) along the entire tributary line
4. Sample (meso)habitat values and riparian zone variables along with elevation data (from elevation raster) using the 1mSP
5. For each 1mSP, sample upstream aggregated data that now are available from 4. with different spatial extents (here we use 50, 100 and 200 m upstream) and format as spatial data frame.

Step 1. Align points to tributary line shape

Due to inaccuracy due to poor satellite reception and/or failure in placing the waypoint correctly on the app map, some points are not perfectly aligned to the tributary line shape objects. As can be seen from the mid panel in **Figure 4** some of the sample points in the close-up are not perfectly aligned to the tributary line shape. By using the R procedure sf::st_nearest_points() new points were aligned to the tributary line as shown in the right panel in **Figure 4**.

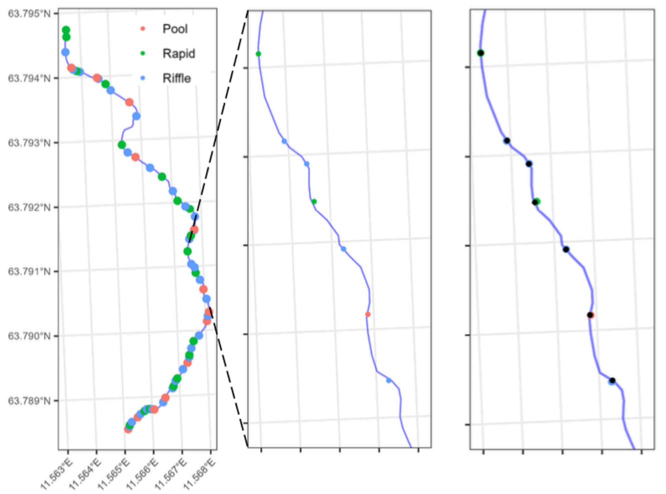

***Figure 4**. Example of mesohabitat assignment points for Korsådalsbekken and how the stream alignment procedure works. Left panel: Raw assignment data; Mid panel: example section where some points are off the stream line; Right panel: aligned points (black) and original points (corresponding to those in the mid panel).*

Step 2. Split tributary line into habitat-specific line segments

Using the new aligned habitat assignment points from Step 1, we split the tributary line into habitat-specific line segments where each assignment point constituted the down-stream starting point for each segment and this segment continued along the tributary line to the intersection point of the next habitat assignment point. This was accomplished by using the R procedure lwgeom::st_split(). The code for Step 1 and 2 is available in **Figure 5** and the resulting data are plotted in mid panel in **Figure 6**.

```
#allign points to stream
nrstMH_KOR = st_nearest_points(KOR_meso, KOR)
on_line_all_MH_KOR = st_cast(nrstMH_KOR, "POINT",do_split=T)
on_line_all_MH_KOR = on_line_all_MH_KOR[seq(2,length(on_line_all_MH_KOR),2),]

#split into habitat segments
buf_all_MH_KOR <- st_combine(st_buffer(on_line_all_MH_KOR,0.01))
parts_all_MH_KOR = st_collection_extract(lwgeom::st_split(KOR$geometry, buf_all_MH_KOR),"LINESTRING")
parts_all_select_MH_KOR=parts_all_MH_KOR[seq(3,length(parts_all_MH_KOR),2),]
parts_all_select_MH_DF_KOR=st_sf(data.frame(mesohab=KOR_meso$MesoHabTyp, geom=parts_all_select_MH_KOR))
online_all_MH_DF_KOR=st_sf(data.frame(mesohab=KOR_meso$MesoHabTyp, geom=on_line_all_MH_KOR))
```

Figure 5. *R code for points-to-stream-line alignment and for splitting into habitat line segments KOR_meso= point shape file with mesohabitat data from Korsådalsbekken, KOR = line shape file for Korsådalsbekken.*

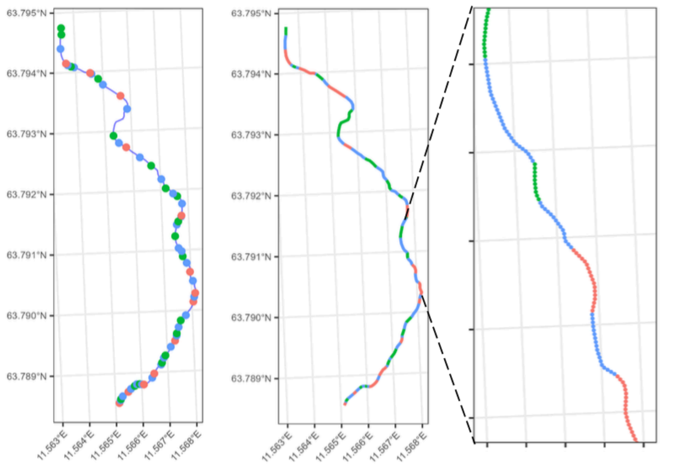

Figure 6. *From raw mesohabitat point assignments (left) to mesohabitat sections (mid) to 1-m resolution mesohabitat points (right).*

Step 3. Establish one-meter sampling points along the tributary line

By using the R procedure sf::st_line_sample() sample points with 1-meter regular resolution (1mSP) was established along the entire tributary lines. We also made an attribute to each of these 1mSP with information on distance to tributary mouth (**Figure 7**). Having these distances at hand will make it easy to attain upstream data for later steps.

```r
#define sampling points (1 m resolution)
samp_KOR=st_line_sample(KOR,density = units::set_units(1, 1/m))
samp_KOR_sf <- st_sf(as.data.frame(samp_KOR),geometry = samp_KOR) %>%
  st_cast("POINT")

samp_KOR_sf$distMouth=1:dim(samp_KOR_sf)[1]
```

Figure 7. *R code for establishing 1mSP along tributary line for Korsådalsbekken and for assigning an attribute with distance to tributary mouth*

Step 4. Sample (meso)habitat values and riparian zone variables along with elevation data (from elevation raster) using the 1mSPs

To sample (meso)habitat values and other line-segment attribute values such as riparian zone variables by using the 1mSPs, the segmented lines from Step 2 had to be rasterized. This was done by developing a rasterization procedure (**Figure 8**). By sampling along these resulting rasterized lines using the 1mSPs (**Figure 9**), the 1mSPs gained multiple attributes including mesohabitat type (see **Figure 6**, right panel) and five riparian zone variables. Finally, after also sampling from a spatial raster with elevation data each 1mSP got an elevation value as well (**Figure 10** and **Figure 11**). The sampled elevation data were derived from lidar scannings and were not cleaned for noise such as road crossings and potentially overhanging obstacles such as large trees. To get more reliable stream elevation profiles, we fit generalized additive models (GAM, using the gam library) that fit smoothed profiles. From the fitted gam models, we could estimate smoothed elevation data (**Figure 11**, right panel). These estimated values were used in further analyses – and for estimating tributary upstream gradients.

At this stage we have created tributary-specific spatial data frames for 1mSP with 10 attributes that represent measured or estimated attribute values each specific 1mSP (see **Figure 12** for an example). The next step is to estimate or sample upstream attributes at various spatial extensions (here, 50, 100 and 200 m upstream).

```r
#prosedure for rasterizing a line
rasterizeLine <- function(sfLine, rast, value){
  # rasterize roads to template
  tmplt <- stars::st_as_stars(sf::st_bbox(rast), nx = raster::ncol(rast),
                              ny = raster::nrow(rast), values = value)

  rastLine <- stars::st_rasterize(sfLine,
                                  template = tmplt,
                                  options = "ALL_TOUCHED=TRUE") %>%
    as("Raster")

  return(rastLine)
}
```

Figure 8*. R code for rasterizing a (segmented) line.*

```r
#sampling meso and RIP-zone-scores
r_RZ_KOR <- raster::raster(parts_all_select_RZ_DF_KOR, resolution=2)
parts_all_select_rast_RZ_KOR <- rasterizeLine(parts_all_select_RZ_DF_KOR, r_RZ_KOR, NA)
r_MH_KOR <- raster::raster(parts_all_select_MH_DF_KOR, resolution=2)
parts_all_select_rast_MH_KOR <- rasterizeLine(parts_all_select_MH_DF_KOR, r_MH_KOR, NA)


KOR_RIPscore = as.data.frame(unlist(raster::extract(parts_all_select_rast_RZ_KOR, samp_KOR_sf)))
samp_KOR_sf=cbind(samp_KOR_sf,KOR_RIPscore)
samp_KOR_sf$mesohab = raster::extract(parts_all_select_rast_MH_KOR, samp_KOR_sf)
samp_KOR_sf$mesohab = as.factor(samp_KOR_sf$mesohab)
```

Figure 9. *R code for rasterizing Korsådalsbekken tributary line segments of mesohabitat assignments and riparian zone variables and then sample from these line rasters with the 1mSPs (here samp_KOR_sf).*

```r
#extract altitude values along stream line
alti_values = raster::extract(altitudeDataKOR, samp_KOR_sf)
samp_KOR_sf$altitude=alti_values$KOR_elevation2
altmod=gam(altitude~s(distMouth),data=samp_KOR_sf)
samp_KOR_sf$estAlt=predict(altmod)
```

Figure 10. *R code for sampling elevation data from a spatial raster file (downloaded from https://hoydedata.no/LaserInnsyn2/) for the surrounding area of Korsådalsbekken using the 1mSPs.*

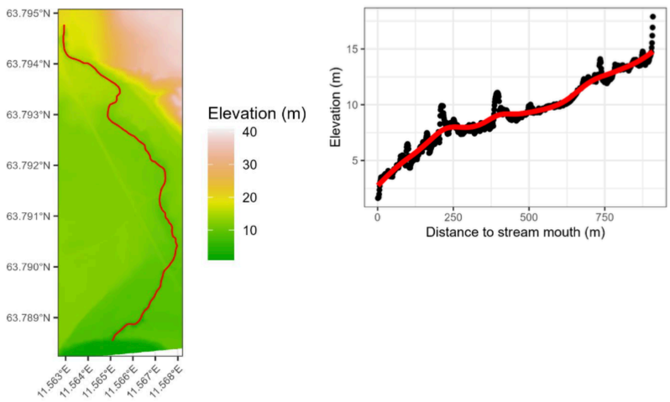

Figure 11. *From raster maps of lidar-derived elevation data (left) to 1-m resolution elevation data (black points) sampled from the raster to GAM-model of elevation profile (red line). Example is for Korsådalsbekken. Elevation rasters were downloaded from https://hoydedata.no/LaserInnsyn2/*

	distMouth	altitude	estAlt	RIP_score	Veg_score	RIP_B_score	Surf_sh_score	Frac_O_score	Frac_L_score	geometry	mesohab
1	1	1.623582	2.759648	NA	NA	NA	NA	NA	NA	POINT (626386.9 7075986)	NA
2	2	1.676373	2.787948	19	5	3	5	5	1	POINT (626387.4 7075987)	NA
3	3	1.676041	2.816248	19	5	3	5	5	1	POINT (626387.9 7075988)	NA
4	4	1.732061	2.844544	19	5	3	5	5	1	POINT (626388.4 7075988)	Pool
5	5	1.980321	2.872831	19	5	3	5	5	1	POINT (626388.8 7075989)	Pool
6	6	2.363703	2.901106	19	5	3	5	5	1	POINT (626389.3 7075990)	Pool
7	7	2.709256	2.929365	19	5	3	5	5	1	POINT (626389.7 7075991)	Pool
8	8	3.008160	2.957603	19	5	3	5	5	1	POINT (626390.1 7075992)	Pool
9	9	3.145636	2.985815	19	5	3	5	5	1	POINT (626390.4 7075993)	Riffle
10	10	3.327319	3.013996	19	5	3	5	5	1	POINT (626390.8 7075994)	Riffle
11	11	3.496157	3.042139	19	5	3	5	5	1	POINT (626391.1 7075995)	Rapid
12	12	3.455339	3.070239	19	5	3	5	5	1	POINT (626391.4 7075996)	Rapid
13	13	3.528415	3.098291	19	5	3	5	5	1	POINT (626392 7075997)	Rapid
14	14	3.504770	3.126289	19	5	3	5	5	1	POINT (626392.7 7075997)	Rapid
15	15	3.387937	3.154229	19	5	3	5	5	1	POINT (626393.3 7075998)	Riffle

Figure 12. *The spatial data frame for 1mSPs in Korsådalsbekken showing all local attributes for each 1mSP.*

Step 5. Add upstream aggregated data attributes to the 1mSP spatial data frame with different spatial extents

Because we already have the 1mSP spatial data frames, upstream aggregated data can be accessed from them directly by just sampling at (to estimate tributary gradient) or between (to estimate aggregated metrics such as mean scores of the riparian zone variables or fraction of running water from the mesohabitat data (i.e., number of 1mSPs with either "riffle" or "rapid" mesohabitat values divided by the spatial extent (100, if 100 m upstream)). An example of how the fraction of running water over a 100 m upstream segment from the 380 m 1mSP in Korsådalsbekken is shown in **Figure 13**. These upstream estimation procedures were performed in a two-level for-loop developed for running through a pooled 1mSP spatial data frame for all tributaries (samp_all_tribs_sf). The first level ran across tributaries and the second loop ran through all rows (i.e., 1mSPs) within each stream (**Figure 14**). The resulting spatial data frame, samp_all_tribs_upstream_sf (Table 1), holds both local attributes pertinent to each 1mSP and all upstream attributes – and this spatial data frame was used to merge into other biological and physical data (fish densities, benthic invertebrates, drift samples, sediment redox values) to explore effects from local and upstream habitat effects on these metrics.

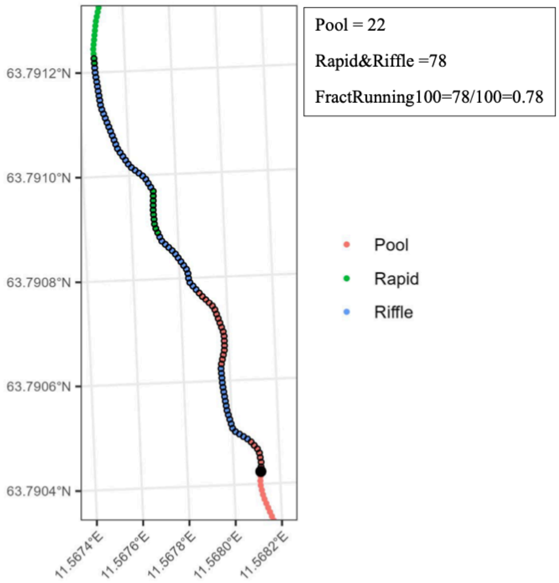

***Figure 13**. Estimation procedure for mesohabitat composition 100 m upstream (black-circled symbols) the 380 m 1mSP upstream tributary outlet in Korsådalsbekken (filled black point). Estimation of fraction of running water for the 100 m upstream is shown in the upper-right frame.*

.

```r
for(i in 1:nlevels(samp_all_tribs_sf$Tributary))   #tributary subsetting
{
  Trib_i_SampData=subset(samp_all_tribs_sf,Tributary==levels(samp_all_tribs_sf$Tributary)[i])
  for(j in 1:dim(Trib_i_SampData)[1])  #upstream estimation at 1-m level within stream
  {
    Trib_i_SampData$alt100[j]=Trib_i_SampData[Trib_i_SampData$distMouth[j]+99,]$estAlt
    Trib_i_SampData$avgRIPscore100[j]=mean(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+99),]$RIP_score,na.rm=T)
    Trib_i_SampData$alt50[j]=Trib_i_SampData[Trib_i_SampData$distMouth[j]+49,]$estAlt
    Trib_i_SampData$avgRIPscore50[j]=mean(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+49),]$RIP_score,na.rm=T)
    Trib_i_SampData$alt200[j]=Trib_i_SampData[Trib_i_SampData$distMouth[j]+199,]$estAlt
    Trib_i_SampData$avgRIPscore200[j]=mean(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+199),]$RIP_score,na.rm=T)
    Trib_i_SampData$fractRunning100[j]=as.numeric(1-table(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+99),]$mesohab)[1]/100)
    Trib_i_SampData$fractRunning50[j]=as.numeric(1-table(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+49),]$mesohab)[1]/100)
    Trib_i_SampData$fractRunning200[j]=as.numeric(1-table(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+199),]$mesohab)[1]/100)
    Trib_i_SampData$avgRIP_widthSc50[j]=mean(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+49),]$RIP_B_score,na.rm=T)
    Trib_i_SampData$avgRIP_widthSc100[j]=mean(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+99),]$RIP_B_score,na.rm=T)
    Trib_i_SampData$avgRIP_widthSc200[j]=mean(Trib_i_SampData[Trib_i_SampData$distMouth[j]:(Trib_i_SampData$distMouth[j]+199),]$RIP_B_score,na.rm=T)
  }
  if(i==1) samp_all_tribs_upstream_sf=Trib_i_SampData else samp_all_tribs_upstream_sf=rbind(samp_all_tribs_upstream_sf,Trib_i_SampData)
  print(paste("Done estimating ",levels(samp_all_tribs_sf$Tributary)[i],". ",nlevels(samp_all_tribs_sf$Tributary)-i," left.",sep=""))
}

#estimating stream gradients
samp_all_tribs_upstream_sf$gradient50=aspace::atan_d((samp_all_tribs_upstream_sf$alt50-samp_all_tribs_upstream_sf$estAlt)/50)
samp_all_tribs_upstream_sf$gradient100=aspace::atan_d((samp_all_tribs_upstream_sf$alt100-samp_all_tribs_upstream_sf$estAlt)/100)
samp_all_tribs_upstream_sf$gradient200=aspace::atan_d((samp_all_tribs_upstream_sf$alt200-samp_all_tribs_upstream_sf$estAlt)/200)
```

Figure 14. *Loop for estimating the upstream attribute values for mesohabitat, riparian zone variables and stream slope (gradient50 aso).*

Table 1. Extract of the attribute table resulting from the entire upstream sampling procedure where each line represents a 1-m resolution point and each column represents point-specific mesohabitat, elevation or riparian zone variable at the given point (columns left to the Tributary column) or 50, 100 or 200 meter upstream. For instance, avgRIPscore50 = the average total riparian zone variable score for the next 50 meters upstream the point in question and fractRunning100 = fraction of the next 100 1-m points with either riffle or rapid mesohabitat (i.e., not pool). The extract is from the 4-35 m upstream the tributary mouth reach in Bjørkbekken (BJO).

x	y	distMouth	altitude	estAlt	mesohab	RIP_score	Veg_score	RIP_B_score	Surf_sh_sco	Frac_O_scor	Frac_L_scor	Tributary	alt100	avgRIPscore100	alt50	avgRIPscore50	alt200	avgRIPscore200	fractRunning100	fractRunning50	fractRunning200	gradient50	gradient100	gradient200
628255	7073661	4	5.64	6.45	Riffle	20	5	4	5	5	1	BJO	7.88	20	7.43	20	9.18	19.19	0.98	1	0.98	1.12	0.82	0.78
628255	7073660	5	5.96	6.48	Riffle	20	5	4	5	5	1	BJO	7.89	20	7.45	20	9.20	19.175	0.98	1	0.98	1.11	0.81	0.78
628255	7073659	6	6.21	6.50	Rapid	20	5	4	5	5	1	BJO	7.89	20	7.46	20	9.21	19.16	0.98	1	0.98	1.10	0.80	0.78
628255	7073658	7	6.19	6.52	Rapid	20	5	4	5	5	1	BJO	7.90	20	7.48	20	9.23	19.145	0.98	1	0.98	1.10	0.79	0.78
628255	7073657	8	6.26	6.55	Riffle	20	5	4	5	5	1	BJO	7.91	20	7.49	20	9.25	19.13	0.98	1	0.98	1.09	0.78	0.77
628254	7073656	9	6.54	6.57	Riffle	20	5	4	5	5	1	BJO	7.92	20	7.51	20	9.26	19.115	0.98	1	0.98	1.08	0.77	0.77
628254	7073655	10	6.90	6.59	Riffle	20	5	4	5	5	1	BJO	7.92	20	7.52	20	9.28	19.1	0.98	1	0.98	1.07	0.76	0.77
628254	7073654	11	7.29	6.61	Riffle	20	5	4	5	5	1	BJO	7.93	20	7.54	20	9.30	19.085	0.98	1	0.98	1.06	0.76	0.77
628254	7073653	12	7.47	6.64	Riffle	20	5	4	5	5	1	BJO	7.94	20	7.55	20	9.31	19.07	0.98	1	0.98	1.05	0.75	0.77
628253	7073652	13	7.79	6.66	Riffle	20	5	4	5	5	1	BJO	7.95	20	7.56	20	9.33	19.055	0.98	1	0.98	1.04	0.74	0.77
628253	7073651	14	8.14	6.68	Riffle	20	5	4	5	5	1	BJO	7.96	20	7.57	20	9.35	19.04	0.98	1	0.98	1.02	0.73	0.76
628253	7073650	15	8.38	6.70	Rapid	20	5	4	5	5	1	BJO	7.96	20	7.59	20	9.37	19.025	0.98	1	0.98	1.01	0.72	0.76
628253	7073649	16	8.32	6.72	Rapid	20	5	4	5	5	1	BJO	7.97	20	7.60	20	9.38	19.01	0.98	1	0.98	1.00	0.72	0.76
628253	7073648	17	7.92	6.75	Rapid	20	5	4	5	5	1	BJO	7.98	20	7.61	20	9.40	18.995	0.98	1	0.98	0.99	0.71	0.76
628253	7073647	18	7.47	6.77	Rapid	20	5	4	5	5	1	BJO	7.99	20	7.62	20	9.42	18.98	0.98	1	0.98	0.98	0.70	0.76
628253	7073646	19	6.98	6.79	Riffle	20	5	4	5	5	1	BJO	8.00	20	7.63	20	9.43	18.965	0.98	1	0.98	0.96	0.69	0.76
628253	7073645	20	6.72	6.81	Riffle	20	5	4	5	5	1	BJO	8.00	20	7.64	20	9.45	18.95	0.98	1	0.98	0.95	0.69	0.76
628253	7073644	21	6.78	6.83	Riffle	20	5	4	5	5	1	BJO	8.01	20	7.65	20	9.47	18.935	0.98	1	0.98	0.94	0.68	0.76
628253	7073643	22	6.78	6.85	Riffle	20	5	4	5	5	1	BJO	8.02	20	7.66	20	9.48	18.92	0.98	1	0.98	0.93	0.67	0.75
628253	7073642	23	6.73	6.87	Riffle	20	5	4	5	5	1	BJO	8.03	20	7.67	20	9.50	18.905	0.98	1	0.98	0.91	0.66	0.75
628253	7073641	24	6.60	6.89	Riffle	20	5	4	5	5	1	BJO	8.04	20	7.68	20	9.51	18.89	0.98	1	0.98	0.90	0.66	0.75
628253	7073640	25	6.54	6.91	Rapid	20	5	4	5	5	1	BJO	8.05	20	7.68	20	9.53	18.875	0.98	0.99	0.98	0.89	0.65	0.75
628253	7073639	26	6.55	6.93	Rapid	20	5	4	5	5	1	BJO	8.06	20	7.69	20	9.55	18.86	0.98	0.98	0.98	0.87	0.65	0.75
628253	7073638	27	6.53	6.95	Riffle	20	5	4	5	5	1	BJO	8.07	20	7.70	20	9.56	18.845	0.98	0.98	0.98	0.86	0.64	0.75
628253	7073637	28	6.47	6.97	Riffle	20	5	4	5	5	1	BJO	8.08	20	7.71	20	9.58	18.83	0.98	0.98	0.98	0.84	0.63	0.75
628253	7073636	29	6.55	6.99	Riffle	20	5	4	5	5	1	BJO	8.09	20	7.71	20	9.60	18.815	0.98	0.98	0.98	0.83	0.63	0.75
628253	7073635	30	6.54	7.01	Riffle	20	5	4	5	5	1	BJO	8.10	20	7.72	20	9.61	18.8	0.98	0.98	0.98	0.81	0.62	0.74
628253	7073634	31	6.56	7.03	Rapid	20	5	4	5	5	1	BJO	8.11	20	7.73	20	9.63	18.785	0.98	0.98	0.98	0.80	0.62	0.74
628253	7073633	32	6.56	7.05	Rapid	20	5	4	5	5	1	BJO	8.12	20	7.74	20	9.64	18.77	0.98	0.98	0.98	0.79	0.61	0.74
628253	7073632	33	6.58	7.07	Riffle	20	5	4	5	5	1	BJO	8.13	20	7.74	20	9.66	18.755	0.98	0.98	0.98	0.77	0.61	0.74
628253	7073631	34	7.03	7.09	Riffle	20	5	4	5	5	1	BJO	8.14	20	7.75	20	9.67	18.74	0.98	0.98	0.98	0.76	0.60	0.74
628254	7073630	35	7.08	7.11	Riffle	20	5	4	5	5	1	BJO	8.15	20	7.76	20	9.69	18.725	0.98	0.98	0.98	0.74	0.60	0.74

References

Harding, J., Clapcott, J., Quinn, J., Hayes, J., Joy, M., Storey, R., Greig, H., Hay, J., James, T., Beech, M., Ozane, R., Meredith, A., & Boothroyd, I. (2009). *Stream Habitat Assessment Protocols for wadeable rivers and streams of New Zealand*.

Appendix 2: Lowest and highest abundance sites with Oligochaeta, Chironomidae, and *Baetis rhodani* counts.

Ten sites with the lowest abundance

Site	Tributary	totAbund	Chiro	Oligo	Brhod
BJØ_1U	BJO	12	4	2	0
BJØ_5L	BJO	28	3	3	2
BRO_1L	BRO	35	5	5	6
FOL_1U	FOL	35	18	7	0
BRO_3U	BRO	38	3	16	5
SKJ_6L	SKJ	38	1	1	12
FOL_3L	FOL	40	14	6	4
SKJ_3U	SKJ	41	1	3	9
BJØ_1L	BJO	49	4	5	16
BJØ_4L	BJO	56	6	16	3

Ten sites with the highest abundance

Site	Tributary	totAbund	Chiro	Oligo	Brhod
FOL_2BL	FOL	307	95	193	1
KORS_3U	KOR	325	74	14	7
BRO_8L	BRO	369	167	64	29
BRO_5L	BRO	371	219	56	1
KORS_2U	KOR	422	75	301	0
BRO_6L	BRO	517	400	43	4
BRO_8U	BRO	568	191	226	73
BRO_5U	BRO	940	608	212	1
KORS_2L	KOR	1132	152	924	0
SKJ_2U	SKJ	1781	1672	18	0

Appendix 3: List of all species found with shorthand names.

Full.name	short.name
Oligochaeta	Oligo
Chironomidae	Chiro
Chironomidae_pupae	ChirPu
Limoniidae	Limoni
Psychodidae	Psycho
Pediciidae	Pedici
Simuliidae	Simuli
Ceratopogonidae	Cerato
Tipulidae	Tipuli
Hydraena	Hydrae
Platambus_Muculatus_Larvae	PlatMuc
Agabus_Larvae	Agabu
Lymnaea_peregra	LymPer
Seracostomatidae	Seric
Rhyacophilidae	Rhyaco
sp_Baetis	Baeti
B_Rhodani	Brhod
Pisidium	Pisid
Centroptilum_luteolum	CentLut
Baetis_Fuscatus	Bfusc
Hygromiidae	Hygro
Elmis	Elmis
Helodes	Elod
B_niger	Bniger
Limnephilidae	Limne
Lepidostomatidae	Lepido
Ptychopteridae	Ptycho
Goeridae	Goerid
Hydrachnidia	Hydrac
B_Vernus	Bvern
Collembola	Colle
Thrips	Thrips
Siphonoperla_burmeisteri	SiphBurm

Diura bicaudata	DiurBica
Coleoptera	Coleo
Steinflue	Pleco
Oreodytes sanmarkii	OreoSan
Stratiomyidae	Strati
Dixidae_pupae	DixiPu
Dryopidae	Dryopi
Gyrinus	Gyrinus
Sommerfugl	Sommer
Chaoborudae	Chaobo
glossiphonia_complanata	GlossCom
Elmidae	Elmidae
Cicadelloidea	Cicade
Fjern_mygg	FjernMy
Haliplus	Haliplus
Bathyomphalus_contortus	BathyCont
Segmentina_nitida	SegNit
Emphemera_danica	EmphDan
Empididae	Empid
Anisus_leucostoma	AniLeuc
Psychomyiidae	Psychomy
Beraeidae	Berae
Haplidae	Haplidae
Brachyptera_risi	BrachRis
Dixidae	Dixi
Diura_nanseni	DiurNan
sp_Perlodidae	Perlo
Scirtidae	Scirti
Limnius	Limnius
Capnopsis_schilleri	CapSchi
sp_ Leuctridae	Leuct
Leuctra_Hippopus	LuecHip
Hydrophilidae	Hydrop
sp_nemouridae	Nemou
Glossomatidae	Glosso
sp_Capniidae	Capnii
Esolus	Esolus
staphylinidae	Staphy

Omphiscola_glabra	OmphGla
Xanthoperla_apicalis	XanApi
Ecnomidae	Ecnomi
Nematocera (mygg)	Nematocera
Trichoptera	Tricho
Baetis_Muticus	Bmuti
Capnia_pygmaea	CapPyg
Diptera_larvae	DipLar
Wormaldia	Worma
Ochthebius	Octhe
Nematode	Nemat
B_macani	Bmaca
Psylloidea	Psyll
Flue	Flue
Chalciodidae	Chalci
Ceratopogonidae_pupae	CeraPup
Hippeutis_complanatus	HippCom
sp_Taeniopterygidae	Taeni
Nemoura_cinera	NemoCin
physidae	Physi
Leuctra_nigra	LeuNig
Simuliidae_pupae	SimuPup
Leuctra_fusca	LeucFus
Clausiliidae	Claus
Asellus_aquaticus	AseAqua
Limnebius	Limneb
Trochulus_hispidus	TrochHisp
Lymnaea_truncatula	LymnTrunc
brachycera	Brachy
Planorbarius_corneus	PlanCorn
curcolionidae	Curco
sp_Nemoura	Nemoura
Nemoura_flexuosa	NemFlex
Leptoceridae	Leptoc
Tabanini	Taba
sp_Chloroperlidae	Chloro
Dinocras_cephalotes	DinoCeph
Polycentropodidae	Polyce

Nemoura_avicularis	NemAvic
Nemoura_viki	NemViki
Capnia_atra	CapAtr
psychodidae_pupae	PsychoPu
Siphlonuridae_amelus	SiphAme
Procleon_bifidum	ProBifi
Amphinemura_sucicollis	AmphSulci
Isoperla_grammatica	IsoGram
nemurelli_pictetii	NemPic
Psocoptera	Psoco
Hydraena_voksen	HydraVok
Syrphidae	Syrph
Ephemura_vulgata	EphVulg

Appendix 4: Summary of SW ranges for each tributary.

Tributary	Mean_SW	Median_SW	Min_SW	Max_SW	SD_SW
BJO	1.859397661	1.910019925	0.89399034	2.323108623	0.415950031
BRO	1.835402176	1.798914204	1.021352784	2.588358897	0.427255299
FOL	1.719137886	1.720333282	0.728711447	2.641563447	0.553524879
KOR	1.423492713	1.420538505	0.659383208	2.175750554	0.624101873
ROS	1.909813814	1.950944956	1.33150301	2.405862333	0.449290215
SKJ	1.679969467	1.8904951	0.376147553	2.43086799	0.629573128

Appendix 5: Summary of ASPT ranges for each tributary.

Tributary	Mean_ASPT	Median_ASPT	Min_ASPT	Max_ASPT	SD_ASPT
BJO	4.782659933	4.909090909	2.666666667	6	0.965870724
BRO	5.294561862	5.107954545	4.142857143	6.6	0.614937572
FOL	4.460507674	4.583333333	2.833333333	6.222222222	0.986819364
KOR	4.189478114	4.308333333	3.666666667	4.5	0.336186203
ROS	4.654807692	4.509615385	4.2	5.4	0.559500825
SKJ	5.315039425	5.324675325	4.571428571	6.066666667	0.481758884

Appendix 6: Summary of abundance count ranges for each tributary.

Tributary	Mean_Abund	Median_Abund	Min_Abund	Max_Abund	SD_Abund
BJO	72.11111111	70	12	141	40.4148625

BRO	260.6875	192.5	35	940	244.9121526
FOL	159.2727273	155	35	307	92.04356676
KOR	414.6666667	296.5	118	1132	365.7647696
ROS	92.25	93.5	77	105	11.52894907
SKJ	240.2142857	96	38	1781	449.9533614

Appendix 7: List of sites with subsample estimates.

Site	Oligogochaeta count	Chironomidae count
FOL_2BL	193	
SKJ_2U	1672	
KORS_1U		147
KORS_2L	924	152
KORS_2U	301	
BRO_5U	212	608
BRO_6L		400
BRO_7L	86	
BRO_7U		134
BRO_8U	226	191