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4317 Master's Thesis

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Influence of Hydromorphology and Freshwater Pearl Mussels (*Margaritifera margaritifera*) on Benthic Invertebrate Communities - In River Hoenselva and River Skorgeelva, Southeastern Norway

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This thesis is worth 60 study points

Summary

Benthic habitats and their benthic fauna, including the freshwater pearl mussel (*Margaritifera margaritifera*), have been investigated monthly in two small rivers in Southeast Norway from June to October 2023. Due to large variations in hydromorphological conditions in running water, 3 different stations were investigated in both rivers, River Hoenselva and River Skorgeelva.

By the Surber sampling method, we found 77 taxa in River Hoenselva and 79 taxa in River Skorgeelva. Dominant taxa groups were Chironomidae larvae (42%), particularly abundant in June, and *Baetis rhodani* (16%). We also performed surface counting of freshwater pearl mussel, as well as substrate digging to investigate potential recruitment.

Average water temperature was equal in both rivers, 14.3°C in River Hoenselva and 15.1°C in River Skorgeelva. There was a significant difference in water velocity between the two rivers, 0.31 and 0.36 m s^{-1} respectively, while highest velocities were observed in June, i.e. 0.59 m s^{-1} in Hoenselva, and 0.49 m s^{-1} in Skorgeelva. During the investigated period, the water depth at the 3 stations in Hoenselva varied between 23.6 – 34.0 cm, compared with 19.0 – 24.0 cm in Skorgeelva. There was no significant difference in substrate index between the two rivers. All 6 investigated riverbed sites were dominated by gravel and cobble. As pH earlier has been documented to be ≥ 6.4 already more then 20 years ago, pH should not be critical for recruitment of freshwater pearl mussels in the rivers. Thus, the redox potential (E_H) in sediments at 5 cm depth were measured and documented significant lower redox potential conditions in Skorgeelva (573 mV), compared to Hoenselva (609 mV), but redox values below 400 mV were never recorded.

According to the NMDS modeling, the temperature, redox potential in the substrate, and the substrate index contributed to grouping of the benthic macroinvertebrate community. Based on further correlation analysis, temperature was significantly negatively correlated with the Shannon index. We also revealed a weak significant positive correlation between redox potential and the Shannon index. Accordingly, the lower redox potential in River Skorgeelva

sediments, might be a contributor to the lower diversity index in this river compared with River Hoenselva.

We were unable to identify a relationship between the density and/or diversity of benthic organisms and the density of freshwater pearl mussels. Due to data limitations, it was not possible to further test the interactions with more advanced statistical models. However, both rivers were classified with good ecological condition based on our results. To better assess the relationship between freshwater pearl mussels and the benthic community, more data would be necessary, preferably from multiple rivers and longer time series. A different approach, such as a stratified study design focusing on areas with high and low mussel densities, could also improve the results.

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Preface

This is a master's thesis at the University of South-Eastern Norway in the field of Technology, Natural Sciences, and Maritime Sciences. We are two students who have collaborated to produce this thesis, and we would like to thank everyone who has contributed to this process.

First and foremost, we would like to extend a big thank you to our supervisors from Norwegian Institute for Nature Research (NINA), Jon Hamner Magerøy and Knut Andreas Eikland, for their patience and valuable advice and support throughout the project. Thank you for the insightful and educational field trips and for lending us the necessary field equipment.

We would also like to express our gratitude to our supervisor at USN, Espen Lydersen, for his assistance during the process. We greatly appreciated that your office door was always open. We would also like to thank the university for lending us field equipment, which has been crucial for conducting our field studies.

A special thank you goes to Elina Lungrin at NINA for her skilled guidance and assistance with species identification in the biology lab in Bø.

Finally, we would like to express our gratitude for the opportunity to write this master's thesis together. This process has been both challenging and rewarding, and we have both experienced considerable academic growth. Collaboration has made the journey more manageable and memorable, and we are proud of the results of our joint work.

May 15th, 2024, Bø In Telemark

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1 Introduction

1.1 River ecosystems

Flowing water is among the most vulnerable and threatened ecosystems on Earth (Dudgeon et al., 2006; Malmqvist and Rundle, 2002; Vörösmarty et al., 2010). Although freshwater covers less than 1% of the Earth's surface (Dudgeon et al., 2006; Gleick, 1998), it is nonetheless highly valuable as it can serve as habitat for a large number of species worldwide. Estimates suggest that around 126 000 plant and animal species are associated with such ecosystems, which is about 10% of all recognizes species (Balian et al., 2008; Garcia Moreno et al., 2014). Today, only a small portion of the world's rivers remains unaffected by human activities, and currently, 65% of habitats associated with flowing water are either moderately or severely threatened (Cumberlidge et al., 2009; Ricciardi and Rasmussen, 1999; Vörösmarty et al., 2010). The main drivers for this degradation are anthropogenic activities, such as damming, channelization, siltation, eutrophication, acidification, and introduction of non-native species. Such factors may lead to altered habitat conditions, reduced dispersal, species and population extinction (Malmqvist and Rundle, 2002; Strayer and Dudgeon, 2010; Wallace et al., 2013).

The biodiversity in freshwater ecosystems provides irreplaceable ecosystem services to humanity, such as food, flood and erosion protection, carbon sequestration and maintaining good water quality through natural filtration and water treatment (Collen et al., 2014; Darwall et al., 2009; Postel and Carpenter, 2012). The well-being of freshwater ecosystems depends on various aspects, including the purity and abundance of water, connections to other parts of the environment, the state of habitats, and the variety of plant and animal species inhabiting them (Karr and Dudley, 1981; Poff et al., 1997). For example, various engineering organisms contribute to altering ecosystem functions in rivers and along riparian zones by influencing the characteristics of the water flow (Butler, 1991; Moore, 2006; Polvi and Sarneel, 2018). Such "ecosystem engineers" thus can create new habitats for other organisms, by directly or indirectly influencing the availability of resources. Such organisms can themselves change their environment only by their physical presence (autogenic engineers), or they can transform material from one form to another, i.e. allogenic engineers (Jones et al., 1994). In rivers, we have several examples of how some species can play an important role for other organisms, through their role as engineers (Polvi and Sarneel, 2018). For example, caddisflies (Hydropsychidae) spin nets between rocks and gravel, stabilizing the substrate and create shelter for other organisms during periods of high water flow (Cardinale et al., 2004). Another example is the mollusks, which may act as substrate for several other organisms, contribute to particle regime and provide as predator shelters (Gutiérrez et al., 2003).

An intact biodiversity can indicate that water-use and ecosystem changes are sustainable (Dudgeon et al., 2006). To ensure ecosystem services and benefits, biodiversity is crucial (Hooper et al., 2005). Ecosystems are significantly impacted by the functional traits of species and the roles they fulfill as dominant species, keystone species, ecological engineers etc. Species interactions implies that the relative abundance of a species may not accurately reflect its importance within ecosystems. Thus, less common species can still exert a substantial influence on ecological interactions (Hooper et al., 2005).

1.2 Ecological indicators

Ecological indicators can be used as an "early warning signal" to assess environmental health and explain observed changes and stressors (Dale and Beyeler, 2001; Harissou et al., 2023; Johnson et al., 2006). Ecological indicators reflect how organisms are affected by pollution (Vergolyas et al., 2020), while water chemistry evaluates nutrient levels and organic matter (HaRa et al., 2019). In rivers, ecological indicators are often more long-term relevant than water chemistry which only provide a snapshot of the current condition (Mobasher et al., 2023; Rosenberg et al., 1986). Although, a combination of both is crucial for comprehensive river health assessments (Atique and An, 2018; HaRa et al., 2019).

In Norway, we are required to evaluate the ecological status of our water bodies, according to the EU Water framework Directive (European Union, 2000), which includes regular waterbody investigations (Energidepartementet, Klima- og miljødepartementet, 2006). In Norwegian rivers, benthic macroinvertebrates are used to evaluate hydromorphological changes, eutrophication and acidification (Direktoratsgruppen vanndirektivet, 2018).

Benthic aquatic macroinvertebrates serve as good indicators for the sampling site, due to their ubiquitous presence, low mobility, relatively long-lived, stable populations. Thus, it is easy to perform quantitative sampling (Reynoldson and Metcalfe-Smith, 1992), and assess disturbances or habitat changes. Accordingly, they are very important for the well-functioning ecosystems, including breakdown of organic matter, food for other organisms, and their role as predators, parasites and saprophages (Rosenberg et al., 1986).

Numeric benthic invertebrate indexes, related to diversity and the sensitivity, are often used to assess ecological condition of rivers (Czerniawska-Kusza, 2005; Zhang et al., 2021). These indexes are often tailored to stress from specific types of organic pollution (Mason, 1996; Stark, 1998). Rivers exposed to little disturbance and stress often have higher diversity in benthic communities, i.e. many species present in relatively small quantities. If the river is subjected to stress, robust species are favored, and sensitive species can be reduced or disappear. Thus, the biodiversity of the river is reduced (Mason, 1996). This can be illustrated by several different indexes, with the Shannon index (Shannon and Weaver, 1949) being one of the most used (Krebs, 1989; Mason, 1996). Another often used index is the Evenness index, telling us something about how well benthic species are distributed in relation to abundance (Krebs, 1989; Wilsey and Potvin, 2000). Typically, this index ranges from 0 to 1, where 1 indicates complete evenness (Kvålseth, 2015).

There are many different indexes mentioned in the literature, most of them based on the British Biological Monitoring Working Party (BMWP) index (Mason, 1996). This system assigns a score between 1 and 10 to various families of benthic macroinvertebrates, based on their sensitivity to pollution, with the total score being the sum of all family scores. One may calculate the average value of these families by dividing the BMWP sum by the number of taxa, named the ASPT index (average score per taxon). If the ASPT index is high, it indicates a large number of sensitive taxa (Armitage et al., 1983). In Norwegian watercourses, the ASPT index is used to assess effects of eutrophication and/or organic load (Direktoratsgruppen vanndirektivet, 2018). Studies by Varnosfaderany (2010) also showed that the ASPT index correlated positively with water quality parameters such as oxygen saturation, water velocity, and pH.

In Norway, we also used the RAMI index (River Acidification Macroinvertebrate Index) to evaluate the presence of macroinvertebrates sensitive to acidification in Norwegian rivers (Direktoratsgruppen vanndirektivet, 2018). RAMI measures the relative abundance of indicator taxa with different tolerance levels to acidification. The index is based on the variation in the species composition and how well they respond to acidification. RAMI is developed by the Northern Intercalibration Group and is based on information from Norway, Sweden, and the UK (Direktoratsgruppen vanndirektivet, 2018 Appendix).

One may also use selected groups of invertebrates, as the EPT taxa evaluation (EPT = Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies)). EPT taxa are sensitive to changes in physical and chemical parameters, as biochemical oxygen demand (BOD), ammoniacal-nitrogen, nitrate-nitrogen, phosphate-phosphorus, conductivity and dissolved oxygen (DO) (Thorne and Williams, 1997) and decreased dissolved oxygen conditions (Mason, 1996). By examining the EPT-community, one can assess the ecosystem's integrity and the degree of impairment. High numbers of EPT taxa are typically found in undisturbed environments with high microhabitat diversity (Vitecek et al., 2021), and with high correlation between abundance and water quality (Ambelu et al., 2010; Selvanayagam and Abril, 2015).

Single species of macroinvertebrates can also be used to determine ecological condition, such as the freshwater pearl mussel *(Margaritifera margaritifera)* used in Norway as a threshold indicator of ecological status in rivers. This species is sensitive to several types of physical, chemical and biological disturbances (Direktoratsgruppen vanndirektivet, 2018). In our thesis, we have used benthic macroinvertebrates, including the freshwater pearl mussel, as indicator species.

1.3 Habitat- and hydromorphological preferences, for the benthic invertebrate community

Macroinvertebrates in river ecosystems exhibit diverse and important functions within their habitats. Their locomotion, feeding behavior, and resource utilization contributes to moving sediment particles in the habitat, an important ecosystem role for these organisms (McCall and Tevesz, 1982; Wallace and Webster, 1996). The concept of functional feeding groups is used to describe groups of benthic organisms based on behavioral mechanisms, such as feeding strategy, along with morphological traits like mouthpart specialization, that they use for resource consumption (Cummins and Klug, 1979). Examples of functional feeding groups are; scrapers, piercers, shredders, collectors, filter feeders and predators (Ramírez and Gutiérrez-Fonseca, 2014). Some examples of the importance of these groups are grazers because they contribute to recycling organic matter (Vanni, 2002), shredders because of breakdown of leaves and subsequent decomposition of organic matter (Cummins et al., 1989). This organic matter can be utilized by collectors residing on the riverbed (Cummins and Klug, 1979), and filter feeders (also called collector-filterers) with different feeding strategies (Ramírez and Gutiérrez-Fonseca, 2014). Functional characteristics of different benthic organisms contribute to maintaining ecosystem services in the watercourse. These traits influence the underlying ecosystem processes that are crucial for interactions between water quality and ecosystem health (de Bello et al., 2010).

To maintain species diversity in the river, it is important to have a certain level of habitat heterogeneity (Harper et al., 1997). Variation in processes such as erosion, transport, and accumulation shape the riverbed, creating a more complex bottom profile and substrate composition, as well as flow refuges and hydraulic dead zones. This provides a variety of available habitats for benthic organisms (Szałkiewicz et al., 2022). Flow and sediment regimes also affect the community structure of benthic organisms in various ways. As an example, erosion is a significant stressor that contributes to reducing species diversity in rivers. Additionally, flow and sediment can alter water quality, which in turn may negatively impact benthic organisms (Xu and Li, 2019).

Running water affects many ecological processes and patterns in rivers (Hart and Finelli, 1999). The hydrological conditions in the river have been found to play a significant role in the variation of species composition, with particular emphasis on water velocity as a key variable (Hart and Finelli, 1999; Trisina and Suen, 2013; Wood et al., 2000). Water flow and velocity can influence a variety of variables, such as habitat, dispersal, resource acquisition, competition and predation (Hart and Finelli, 1999). Benthic macroinvertebrates exhibit varying preferences regarding hydraulic habitats. Residing in turbulent environments offers advantages such as enhanced access to food and oxygen, albeit at the expense of higher energy expenditure (Wiley and Kohler, 2011). Some taxa like Spinygilled mayfly (*Coloburiscus humeralis)* prefer higher velocity, >0.75 m s^{-1}, and some species like the freshwater pearl mussel like somewhat lower velocity, 0.25 - 0.75 m s^{-1} (Hastie et al., 2000).

Many species of benthic macroinvertebrates typically reside in the substrate where flow conditions are favorable for them (Hart and Finelli, 1999). Several species exhibit substrate preferences, typically favoring gravel and coarser materials. A study from New Zealand in 1990 examined 12 taxa of macroinvertebrates across four rivers, revealing that none of the taxa exhibited a preference for fine substrates such as sand and fine gravel, or deep water (Jowett et al., 1991). Trisina and Suen (2013) found that the number of species was negatively correlated with water depth.

EPT taxa have shown to have clear habitat preferences and are significantly affected by dissolved oxygen and temperature regime, in addition to eutrophication. Particularly, Plecopterans are very sensitive to these parameters (Hrovat et al., 2014). Basaguren and Orive (1990) observed changes in the structure of the Trichopteran communities with different disturbances, as nutrient level and dissolved oxygen conditions. Trichopterans disappeared at low dissolved oxygen levels.

Benthic organisms are influenced by various environmental conditions, such as dissolved oxygen, temperature, food availability, and the activity of other organisms. Many taxa, as oligochaetes, insect larvae, crustaceans, and bivalve mollusks, are also capable of altering their habitat due to their activities related to movement and feeding (McCall and Tevesz, 1982). For example, Shang et al., (2013) discovered that chironomids larvae inhabiting the sediment exerted a substantial influence on augmenting the oxygen content within their habitat.

1.4 The freshwater pearl mussel

One of the focal species in this thesis is the freshwater pearl mussel. This mussel is assessed as endangered globally, critically endangered in Europe by the International Union for

Conservation of Nature (IUCN), and vulnerable according to the Norwegian Red List (Bakken et al., 2021; Moorkens, 2010; Moorkens et al., 2010). Due to these statuses and the protections provided under legislation, conservation efforts are crucial to safeguard this species (Boon et al., 2019). The freshwater pearl mussel is also used to assess the ecological quality of rivers in Norway (Direktoratsgruppen vanndirektivet, 2018).

The freshwater pearl mussel is a filter feeder, which can purify up to 50 liters of water a day (Larsen, 2005; Moorkens, 1999). The mussel is long-lived, some studies indicating up to 200 yrs (Dolmen and Kleiven, 1997a; Larsen, 2005), while other studies indicate up to 300 yrs in Scandinavia (Degerman et al., 2009). It is dependent on either Atlantic salmon (*Salmo salar*) or trout (*Salmo trutta*) as hosts for their larvae (glochidium), as they have a parasitic stadium on the fish gills, which lasts for ≈ 7 months (Dolmen and Kleiven, 1997a; Larsen, 2005; Moorkens, 1999). Small mussels then live buried in the substate of the riverbed for ≈ 4-8 yrs (Dolmen and Kleiven, 1997a; Larsen, 2005). The small mussels (<50 mm), living in the substrate, are a sign of a recruiting population (Geist and Auerswald, 2007). When the mussel is large enough, it comes up to the surface, where it stands with its head into the substrate and backend into the water column (Dolmen and Kleiven, 1997a; Larsen, 2005). After 10-15 yrs the freshwater pearl mussel is ready to start reproducing, and continue reproducing throughout its life (Larsen, 2005).

The freshwater pearl mussel is quite selective regarding habitat preferences (Larsen et al., 2000). Factors as water velocity, depth, river morphology and substrate affect the mussel (Magerøy et al., 2020). It prefers water velocity values between 0.25-0.75 m s^{-1} (Hastie et al., 2000, summarized in Degerman et al., 2009; Larsen, 1997; Quinlan et al., 2015). High velocities (>0.3 m s^{-1}) will result in unfavorable habitats for young mussels (Larsen, 2017). Overall, it prefers shallow areas, often near the riverbanks (Hastie et al., 2000; Varandas et al., 2013), but it is documented that mussels are often found at depths ranging from 0.1 to 2 m (Hastie et al., 2000, summarized in Degerman et al., 2009).

Regarding the substrate, the freshwater pearl mussel seems to prefer habitats that consists of large rocks and boulders that create stability and refugia full of sand and gravel (Hastie et al., 2000). To be able to bury into the substrate, the mussel depends on sandy habitat, and boulders to stabilize the substrate (Larsen, 1997). Adult mussels can also be found in habitats that are muddy or full of silt for an unknown period, but juvenile mussels are not found in these habitats. Too much fine sediments clog the pores of the substrate, reducing the exchange of oxygen rich water. Thus, juvenile mussels dependent on favorable oxygen conditions in the substrate will likely not survive (Magerøy et al., 2020). Regarding the juvenile mussel oxygen demand, it is vital that the redox potential is close to equal between the water column and substrate (5-10 cm). Sites with substantial difference in redox potential between the water column and the substrate are associated with limited recruitment (Geist and Auerswald, 2007).

The freshwater pearl mussel is a unique species that to some degree, can be described as a flagship species in freshwater ecosystems (Geist, 2010). It is not considered as charismatic as other large vertebrate flagship species, but the mussel is often associated to clean waters (Geist, 2005). The mussel also has a long and important history in Europe because of their valuable pearls, which were used as ornaments and gifts among royals or other high-ranking individuals. The interest in pearls dates back to ancient times. In the Nordic countries, the royals took control of all pearl fishing and had exclusive rights to all pearls found. Later, the rights to pearl fishing were transferred to the landowners. Pearl fishing was common practice until the middle of the 1900s (Larsen, 1997), when the cultured pearls took over the market (Nagai, 2013). It wasn't until 1993, when the freshwater pearl mussel was protected, that the harvest of the species was made illegal, although some cases of hobby-pearl fishing could still be documented in 1997 (Dolmen and Kleiven, 1997a). It is safe to say the mussel has played an important part of European history and art (Larsen, 1997), and using it as a flagship species only heightens its value and importance.

For oligotrophic rivers, the freshwater pearl mussel is a key indicator species (Boon et al., 2019; Geist, 2010). They are well adapted to oxygen-saturated waters, with generally low water temperatures and low concentrations of nutrients and lime (Geist, 2005). Also, they are an indication of undisturbed habitats, both for headwater regions and smaller streams (Geist and Kuehn, 2005). They are also suitable indicators for well-functioning and healthy ecosystems with few changes in water quality, and cooccurrence of other specialized species

(Geist, 2005). In addition, they are relatively easy to identify, and are found within a wide geographical range, and can therefore be used as an indicator across nations (Geist, 2010). Therefore, this species can be used to assess the ecological condition of rivers, making it cheaper and easier to obtain a relatively comprehensive picture over the ecosystem's health (Brito et al., 2018). In Norway, this species is used as a threshold indicator to evaluate the ecological status of watercourses, partly because of their sensitivity to acidification and disturbances (Direktoratsgruppen vanndirektivet, 2018).

The freshwater pearl mussel also fits the criteria of an umbrella species (Geist, 2010). Umbrella species are selected because of their large home ranges, which makes the conservation efforts made to protect them protects many other species (Geist, 2005). For freshwater pearl mussels, it's not only their home range that matters, but also their dependence on the surrounding areas of the rivers being kept in good condition. Not only factors in the river *per se*, but also river catchment geology, as well as land use in the catchment area are important (Strayer et al., 2004). Hence, a conservation program for freshwater pearl mussel must cover challenges within the whole river catchment. Accordingly, freshwater pearl mussel conservation does also protect many other species that live in the catchment areas, including river-cohabitants (Geist, 2010).

The freshwater pearl mussel is also an important keystone species (Geist, 2010), because its presence can change the aquatic ecosystem (Geist, 2005). One way they alter the ecosystem is the ability to filter up to 50 liters of water every day, decompose organic matter and deposit the inorganic matter on the substrate (Larsen, 2005). This filtering removes organic matter and inorganic matter from the water column, clearing the water and increasing the light penetration (Vaughn and Hakenkamp, 2001). More light means more macrophytic plants and epiphytes which can be used for attachment, food, and cover for other river-dwelling species (Geist, 2010). In rivers that are minimally impacted by nearby agricultural areas, mussels create local biodiversity hotspots through nutrient excretion (Spooner et al., 2013). Eveleens et al., (2023) uncovered in a recent study a significant relationship between freshwater mussels in general and the benthic community. Among other findings, they discovered that the richness of mussel species and the presence of threatened mussel species positively correlated with the diversity in the macroinvertebrate community.

Through filtering of the water column, the freshwater pearl mussel deposit feces and pseudofeces into the substrate (Vaughn and Hakenkamp, 2001). Pseudofeces is the inorganic component expelled without passing through their digestive system, while feces is the organic component (Larsen, 1997). Depositing into the substrate makes the organic and inorganic components available as nutrients for other benthic macroinvertebrates living in the substrate (Larsen et. al., 2012). Freshwater mussels can have a strong impact on the composition of the benthic invertebrate community, partly because they affect the availability of resources such as chlorophyll a and organic material, through various processes such as nutrient excretion and biodeposition. Additionally, the mussel itself can function as a substrate where other bottom-dwelling organisms can find refuge. It has been shown, for example, that living mussels have a greater number and greater diversity of benthic organisms on and around their shells (Vaughn et al., 2008). The macroinvertebrates benefitting from the nutrients, will in turn become an important food source for fish (Hastie and Young, 2003).

Another important quality of the freshwater pearl mussel is that it causes changes in the structure of the riverbed substrate, as well as being food for other organisms, structures to attach to and a place for cover (Geist, 2005). Burrowing activities also increase waterflow through the substrate, and increases the oxygen content, making the habitat more available to other species (McCall et al., 1979; Spooner and Vaughn, 2006). Combining the filtering, bio deposition and burrowing in the substrate, the freshwater pearl mussel is an important keystone species for the areas in which they inhabit.

1.5 Aim of the study

Since rivers are important ecosystems with high species diversity and are highly vulnerable, it is important to preserve them (Vörösmarty et al., 2010). Ecological indicators can be used as an early warning system to assess ecological condition, as well as changes that occur over time (Dale and Beyeler, 2001). In Norway, indexes for benthic macroinvertebrates are used to evaluate ecological condition in rivers. This is also evaluated by use of threshold indicator species, such as the freshwater pearl mussel (Direktoratsgruppen vanndirektivet, 2018).

Benthic macroinvertebrates are affected by various factors such as water velocity, oxygen content in the water, nutrient levels, as well as changes in habitat, eutrophication, and acidification (Direktoratsgruppen vanndirektivet, 2018; Hart and Finelli, 1999; Hrovat et al., 2014; Rosenberg et al., 1986). Different compositions of benthic macroinvertebrate communities reflect different habitat preferences and can indicate the condition of the habitat (Rosenberg et al., 1986). A recruiting population of freshwater pearl mussel may serve as a good indicator for ecological water quality (Direktoratsgruppen vanndirektivet, 2018).

In our study, we have looked at how hydromorphological parameters and the freshwater pearl mussel density affect the benthic macroinvertebrate fauna. Our main hypothesis is that hydromorphological parameters and freshwater pearl mussels affect the benthic macroinvertebrate fauna in rivers. Accordingly, we have investigated several variables as abundance and density within the benthic macroinvertebrate fauna and tried to link this to several hydromorphological variables as water velocity, depth, redox potential, substrate roughness, and temperature. Additionally, we wanted to examine the interactions between the freshwater pearl mussel and benthic macroinvertebrates. Finally, we also wanted to assess ecological status of the investigated rivers, River Hoenselva and River Skorgeelva.

2 Material and methods

2.1 Study area

Figure 1 Area of interest. 1 = River Hoenselva, 2 = River Skorgeelva.

The intention of this study was to examine four rivers, however two of them had be excluded. The first river to be excluded was River Svarthølbekken because of its very monotonous substrate, consisting mostly of sand and fine gravel. The second, River Skoeelva, were excluded partially because of workload management and its low densities of freshwater pearl mussels. The two selected rivers were then: River Hoenselva and River Skorgeelva. They are both located in the southeastern part of Norway, Hoenselva in Øvre Eiker Municipality in Buskerud County, and Skorgeelva in Sandefjord Municipality in Vestfold County (**Figure 1**). These rivers were selected because we needed to have extensive background information on the rivers for our study. Since both rivers are a part of the national monitoring program for the freshwater pearl mussel, there was a lot of information to be found. We also needed the river to contain large populations of mussels, as our initial goals included studying the impact of hydromorphological parameters on mussel recruitment. We also wanted to study how the benthic invertebrate fauna interacted with the mussel fauna, so we needed the rivers to have habitats that would give a varied invertebrate fauna.

2.1.1 River Hoenselva

2.1.1.1 Study area

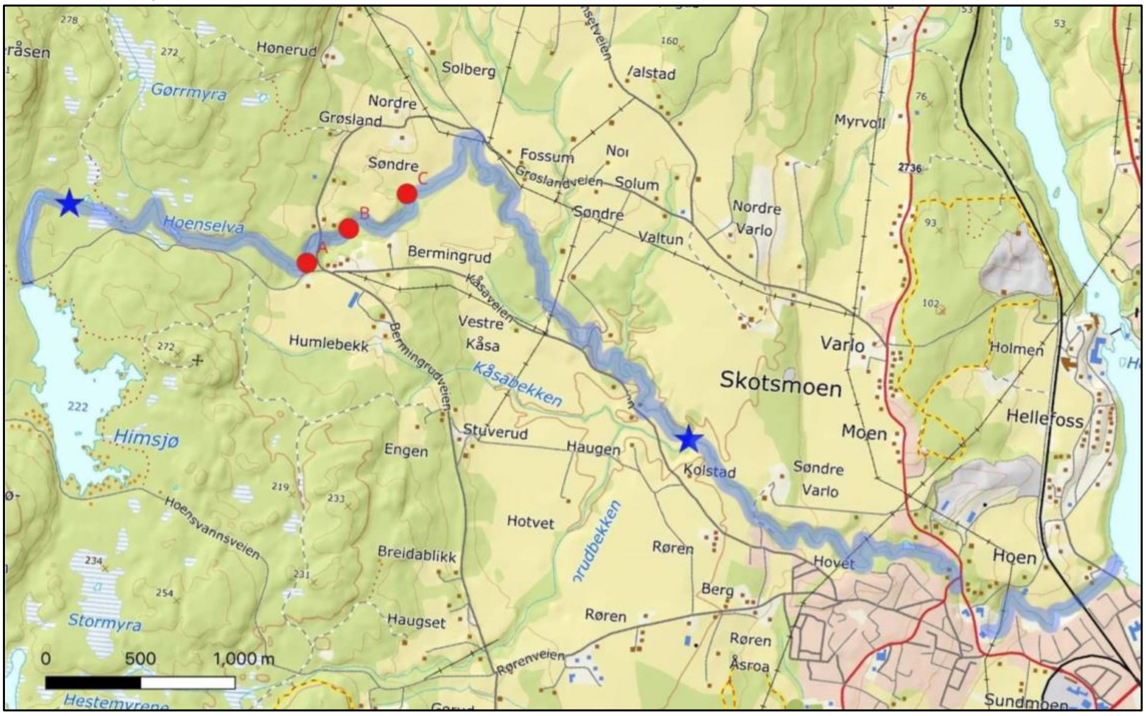

Figure 2 Overview of River Hoenselva, marked in blue, in Øvre Eiker municipality. The river reach delineated by the blue stars represent the known distribution of the freshwater pearl mussel (*Margaritifera margaritifera*). Stations examined in this study are denoted by red dots.

River Hoenselva (watercourse number: 012.B2Z, nve.nevina) is a tributary of River Drammenselva. It is approximately 8 km long, starting in Lake Himsjø and ending up in Drammenselva just north of Hokksund (Larsen, 2002). Hoenselva has some tributaries, like the Stream Kåsabekken, that enter Hoenselva above Varlo (**Figure 2**). This is also around where the mussel distribution ends. Hoenselva traverses through a coniferous forest region, and upon reaching the marine boundary, its course primarily meanders through cultivated lands. This renders its lower reaches more prone to runoff and erosion from agricultural areas (Larsen et al., 2002). Additionally, the riverbed accumulates more clay deposits in these reaches. The catchment area of Hoenselva is about 44 km^{-2} (**Figure 3**), with a runoff of 16.5 l/s*km^{-2} (Norwegian Water Resources and Energy Directorate, nevina.nve.no). The water flow in Hoenselva undergoes significant fluctuations throughout the year and is notably susceptible to the impact of droughts or intense rainfall events (Larsen and Magerøy, 2019). The catchment area receives approximately 390 mm of precipitation during the summer and 430 mm during the winter (Norwegian Water Resources and Energy Directorate, nevina.nve.no).

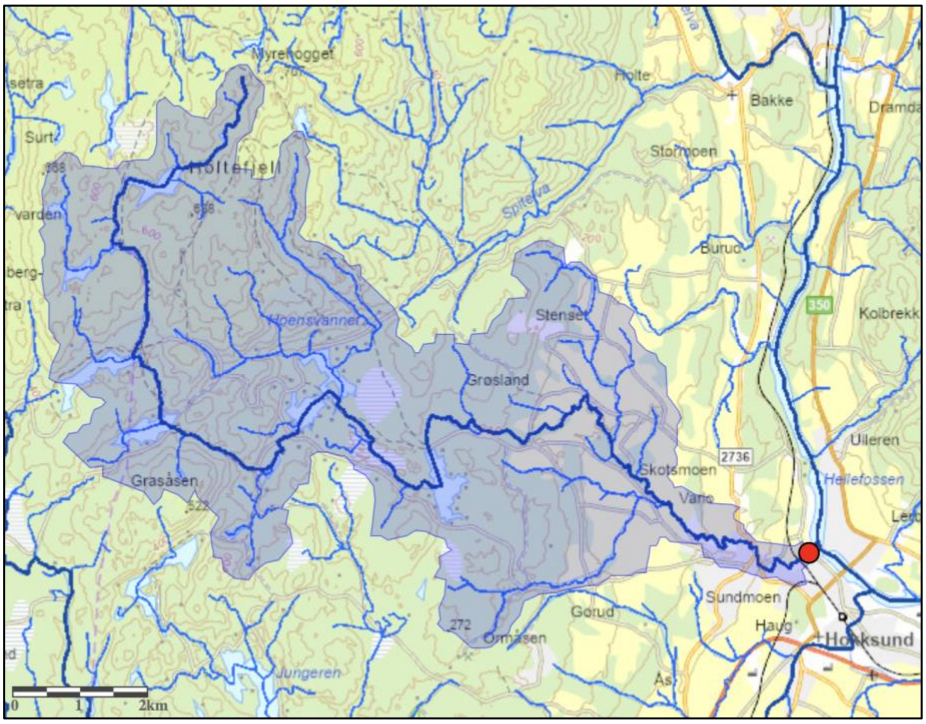

Figure 3 River Hoenselva´s catchment area. Retrieved from the Norwegian Water Resources and Energy Directorate, nevina.nve.no.

River Hoenselva is situated in lowland regions (<200 m above sea level) within the Østlandet ecoregion. It is distinguished by low calcium levels and a high humic content in the upper reaches of the watershed, above Bermingrud. Moving downstream, the river transitions to a moderately calcium-rich and a high humic content river (Larsen and Magerøy, 2019).

Weather data was retrieved from seklima.met.no (https://seklima.met.no/observations/) for the period from May to October 2023. We used weather data from Hokksund, which was the nearest weather station. This area received 525.6 mm of precipitation during our study season, with a noticeable peak in August (196 mm) (**Figure 4**). The high level of precipitation is partially explained by the extreme weather "Hans", which hit southern Norway from the 6th to the 9th of August (Granerød et al., 2023). The average temperature was 13.6 °C, with a peak in June (18,6°C) (**Figure 4**).

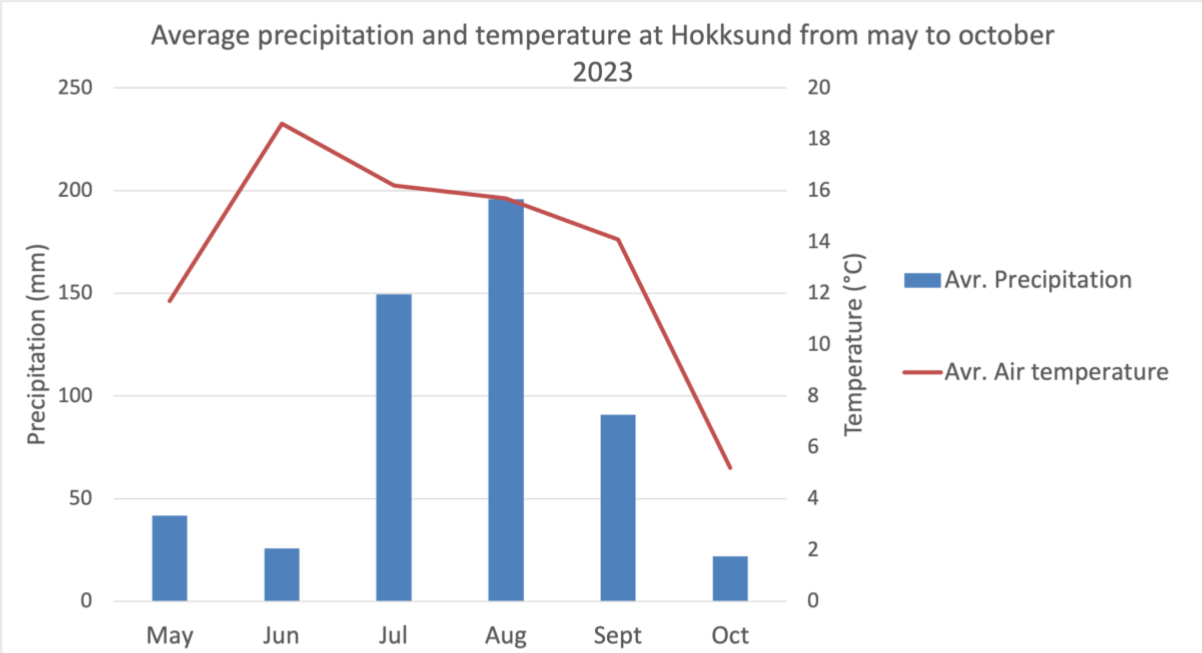

Figure 4 Temperature (°C) and precipitation (mm) measurements for the Hokksund area during the study period. Retrieved from: https://seklima.met.no/observations/

2.1.1.2 Water quality in River Hoenselva

Larsen et al., (2002) researched the freshwater pearl mussel and fish stocking in River Hoenselva in 1996-97. They also surveyed the water quality of the upper part of the river, at Bermingrud. They found that the pH in the upper reaches of the river was an average of 6.6, which they found reflects the high alkalinity, as well as the high concentration of calcium (2.94 mg L^{-1}). They also found that Hoenselva had a moderately high watercolor (38 mg Pt L^{-1}), due to the natural runoff from surrounding forests. The turbidity was measured to 1.5 FTU, showing that the river has moderate to high turbidity during certain periods. As for the nitrate concentration, the average measured in Hoenselva was 77 µg L^{-1}. This classified Hoenselva with a 'good' ecological condition when it comes to the amount of nitrogen in the river (Direktoratsgruppen vanndirektivet, 2018).

The national monitoring program of the freshwater pearl mussel in Norway, published a report by Larsen (2002). This report also included data from another part of the river called Varlo, which is located in the lower reaches of the river. Here, they did further analyses of the nitrate concentration. They found that the highest concentrations of nitrates in the upper part of the river were around 200 μg L^{-1}, while the highest concentrations in the lower reaches were almost 500 μg L^{-1}. This shows a substantial difference in water qualities in the same river. The average concentration of total phosphorous was 3.5 μg L^{-1} in the upper reaches and 11.1 μg L^{-1} in the lower reaches, which is moderately high. Based on this, we see that River Hoenselva has varying water quality between upper and lower reaches.

The water quality in River Hoenselva was further examined in 2008, during the national monitoring program for freshwater pearl mussels in Norway, by Larsen and Berger (2009). They summarized the results from previous measurements of water quality. The pH was about the same as in 2001 in Bermingrud, and in Varlo it was around 6.7 to 7.5. The calcium concentration was higher at Varlo (5.8 mg L^{-1}) compared to Bermingrud (2.7 mg L^{-1}). The alkalinity was much higher at Varlo than at Bermingrud, and the watercolor was measured to be moderately high (42 mg Pt L^{-1}) at Bermingrud, slightly higher than in 2001. The turbidity was an average 0.64 FTU at Bermingrud and 1.80 at Varlo, showing a substantial difference between the two reaches. They reasoned that the difference between stations is due to more clay rich ground, as well as the agricultural lands downstream. The average nitrate concentration at Bermingrud from 1996 to 2008 was 46 μg L^{-1}, with the highest value being 197 μg L^{-1} in 1997, summarized by Larsen and Berger (2009). The concentrations were much higher at Varlo, with values up to 1300 μg L^{-1} in 2008. At Bermingrud, the concentration of phosphorous varied from 1.9 to 3.5 μg L^{-1} from 2001 to 2008, while no concentrations were under 5 μg L^{-1} at Varlo. Thus, the water quality is better in the upper reaches of the river and worse in the lower reaches.

Larsen (2017) summarized the results from the national monitoring program of freshwater pearl mussel from 1999 to 2015 including water quality in River Hoenselva. The results from this report are averages of 18 measurements at Bermingrud, and 11 measurements at Varlo from the period 1999-2010. The average pH was 6.7 at Bermingrud and 7.01 at Varlo. The turbidity was on average of 0.64 FTU at Bermingrud and 1.82 FTU at Varlo. The calcium concentrations were on average 2.67 mg L^{-1} at Bermingrud and 5.57 mg L^{-1} at Varlo. There was a huge difference in the nitrate concentrations between the two stations, with 44 μg L^{-1}

at Bermingrud and 491 $\mu g\ L^{-1}$ at Varlo. There was also an increase in total phosphorous at Varlo, with an average of 8.7 $\mu g\ L^{-1}$, while at Bermingrud the average was 2.8 $\mu g\ L^{-1}$.

Comparing the studies conducted in 1996-97 and 2001 to the one in 2008, we see that not much has changed for the water quality. There was a slight increase in nitrate and phosphorous concentration between those studies, mostly in the lower part of the river (Larsen, 2002; Larsen et al., 2002; Larsen and Berger, 2009). The nitrate content classified River Hoenselva as good in the upper part, but the lower part is classified as poor (Larsen and Berger, 2009). The summary from 2017 also added to the fact that there is a substantial difference between the upper part and the lower part of the river (Larsen, 2017).

Water chemistry samples were not taken during our studies in River Hoenselva. This was partly due to workload management and because samples had already been taken in the same areas during previous investigations over a ten-year period (1999-2010) (Summarized in Larsen, 2017).

Larsen et. al., (2012) conducted a study of the habitat quality for juvenile freshwater pearl mussels in River Hoenselva in 2011, measuring the redox potential in the river. They found that two of the stations in the lower part of the river had some measurements under 300 mV in the substrate. The upper station showed somewhat higher values, with no measurements under 300 mV. This means that the habitat quality was better in the upper part, and that it declines as you move downstream. However, they also found some patches in the lower part that had higher redox potential (above 400 mV). Larsen and Magerøy (2019) also conducted a study on the redox potential in Hoenselva. This time, only one station had values below 300 mV. Values below 300 mV is not sufficient for the freshwater pearl mussels living in the substrate (Geist and Auerswald, 2007). In the lower part of the river, only patches with good redox potential were found in the substrate, while the stations upstream had higher values (median between 450-500 mV). This gives better living conditions for the freshwater pearl mussel, as well as the benthic invertebrate fauna in general (Geist and Auerswald, 2007; Knott et al., 2019), and the habitat quality is 'moderate' to 'good'. This reflects the differences in water quality within the river, which shows that the water quality is 'good' in the upper part of the river, but bad in the lower part of the river (Larsen and Magerøy, 2019).

2.1.1.3 Freshwater pearl mussels in River Hoenselva

Larsen (2002) studied the freshwater pearl mussel population in River Hoenselva. Freshwater pearl mussels are found within a reach of 6.2 km, from the outlet of Vesledam to the confluence of Hoenselva with Stream Kåsabekken (**Figure 2**). They found mussels at every station within this area. The density of freshwater pearl mussels in 2001 was 2.18 individuals per m^{-2}, and they found that there were higher densities in the upper part of the river. They also calculated the population size based on a total river area of 34 000 m^{-2} and density of 2.18 individuals per m^{-2}, as well as accounting for the mussels that are buried in the substrate. This gave a population size of 94 000 individuals in Hoenselva.

The recruitment was found to be limited. Of the mussels they measured, only 1% was under 6-8 yrs, and 6% was younger than 12-13 yrs. This means that there has been recruitment over the last 20 years, albeit lesser than expected (Larsen, 2002). However, glochidia were found on trout through the entire year in 1997 (Larsen et al., 2002), although there was a slight decline during the year, which is to be expected. The ones that were attached got incapsulated on the gills and had normal growth. The number of glochidia varied a lot from 1996 (average 315 glochidia on one side) to 1997 (average 45 glochidia on one side). Still, the infection shows that the mussels use trout as a host fish. The salmon had a few glochidia on them, but they all fell off before they could get incapsulated, showing that they are not the preferred host fish for the freshwater pearl mussel in River Hoenselva.

A similar study was conducted in 2008, in the same study area (Larsen and Berger, 2009). This time, the densities of freshwater pearl mussels were measured to 1.87 individuals per m^{-2}, with the highest densities in the upper part of the river, similarly to 2001. They found a total density of 1.87 individuals per m^{-2}, giving a population size of 63 400 individuals in the river. They also found no mussels younger than 20 years old in the lower part of the river, showing that the recruitment still was little to nonexistent there. However, in the upper reaches of the river they found that one fourth of the population were 20 years or younger, showing that this part of the river had a viable population. When it came to the glochidia on fish gills in this study, they found that the number was lower than expected in most of the river, except from at Bermingrud. At Bermingrud, about half of the trout had glochidia on their gills (average 91 glochidia), which is a moderate intensity of glochidia. The other stations had little to no fish and glochidia. There were no glochidia found on the salmon during this study.

Freshwater pearl mussel surveys conducted by Larsen and Magerøy (2019) revealed a density of 2.09 individuals per m^{-2} in the same area, with the highest density observed in the upper part of the river. They also found that the recruitment was better in the upper part of the river, and that the lower reaches had little to no recruitment. Based on the surveys from 2001, 2008 and 2018, we see that the mussel habitat is better in the upper reaches of River Hoenselva and that the population in the lower reaches of the river is in decline. They did not examine the trout for glochidia in 2018. The lack of recruitment in the lower reaches shows that the freshwater pearl mussel population is not viable, and that its future is uncertain (Larsen and Magerøy, 2019).

2.1.1.4 Benthic macroinvertebrates in River Hoenselva

To our knowledge only one survey of the benthic invertebrate community exists from River Hoenselva. Johansen (1990) did a survey where they gathered the benthic macroinvertebrates that were suspended in the water column, using a drift sampler in the months May to October in 1989. They used two stations, one in the upper part of the river, and one about 4 km further down the river. At the upper station, the first four months were dominated by black flies (Simuliidae), Chironomidae and other Diptera species, and October was especially dominated by the black flies. For the lower station, June was dominated by terrestrial insects. July, September, and October were dominated by the black flies, and August was dominated by Chironomidae.

To find out more about the benthic invertebrate community in River Hoenselva, we used Brittain et al. (1985), which is a report from River Drammenselva and its tributaries. We chose River Bingselva, because it is another tributary of Drammenselva, like Hoenselva. Bingselva is similar to Hoenselva with respect to the substrate, with larger rocks laying on gravel (Brittain et al., 1985), as well as the presence of freshwater pearl mussels (Larsen et al., 2002). Brittain et al. (1985) found that the Drammenselva contained more families of benthic organisms, but the Bingselva had more families that were less tolerant to acidification. This means that the benthic fauna of the Bingselva was less diverse, but also less affected by acidification. There were larger numbers of stonefly species (Plecoptera) in Bingselva compared to the Drammenselva. They also found that Drammenselva watercourse in general had few black fly (Simuliidae) species, with Bingselva being one of the few stations that had such species.

2.1.1.5 Fish populations in River Hoenselva

In River Hoenselva, the fish population consists of Atlantic salmon, trout, minnow (*Phoxinus phoxinus*), and lampreys (Petromyzontiformes) (Larsen et al., 2002). An important factor in the fish population in this river is that there has been released salmon fry from the late 1960s. This is because Hoenselva is a tributary of River Drammenselva, which is infected with the parasite *Gyrodactylus salaris*, making the salmon population decrease drastically. Therefore, the release of salmon was an important measure to help the population recover. In 1993 to 1998 they released about 25 000 to 75 000 salmon fry yearly in Hoenselva, amounting to about 245 000 individuals. In Larsen et al., (2002) survey, they found a sufficient number of salmon, but the trout population occurred in lower densities than expected. The trout that they did find, had good growth rates and they found individuals up to seven years old. Larsen and Berger (2009) did further studies in 2007 and found very low densities of trout throughout the study area. Only a few trout fry were found at the stations, while one station by Fossum had no trout at all. However, this station was the one with the most salmon fry. Salmon fry was found in sufficient numbers at all stations. In 2017, the release of salmon fry was halted in Hoenselva (Larsen and Magerøy, 2019). Larsen and Magerøy (2019) studied the fish population in Hoenselva again, and the density of the trout population was still low in the entire river. It is assumed that the trout population need more time to repopulate after the extensive release of salmon fry, as the salmon has dominated their habitats.

2.1.2. River Skorgeelva

2.1.2.1 Study area

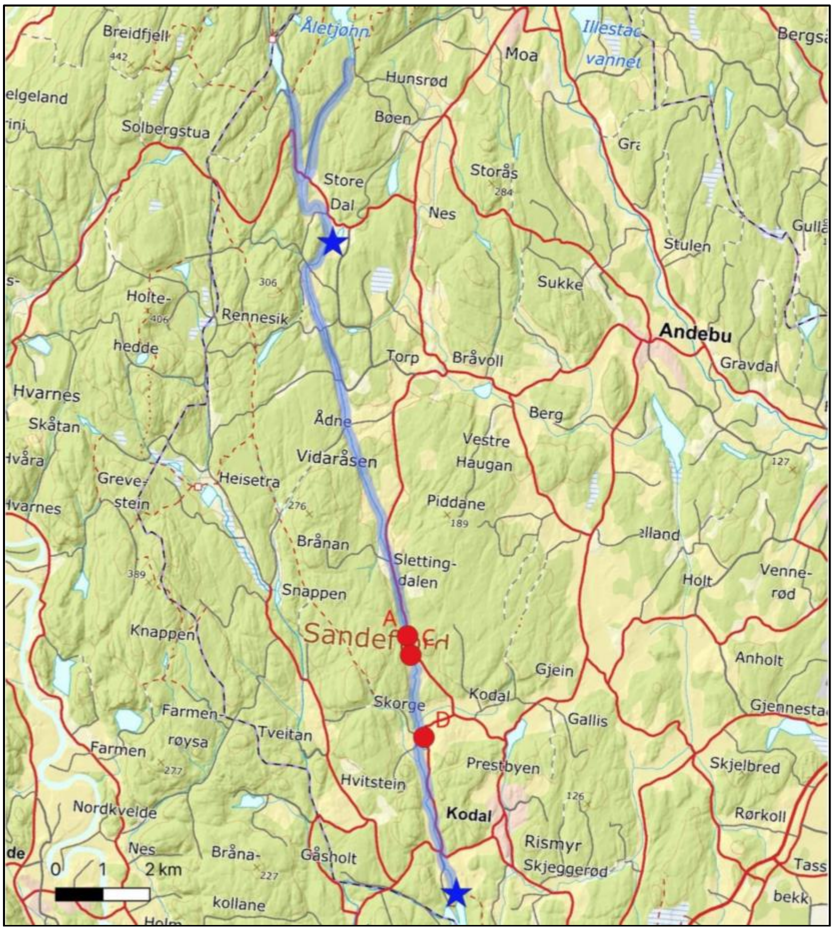

Figure 5 An overview of River Skorgeelva, in Sandefjord municipality. The reach delineated by the blue stars represent the known distribution of the freshwater pearl mussel. Stations examined in this thesis are denoted by red dots. Station B was omitted and thus does not feature on the map.

River Skorgeelva belongs to the Østlandet ecoregion and is located in lowland areas (<200 meters above sea level), characterized as having low calcium levels and being clear (or humic). Skorgeelva (watercourse number: 015.ADZ, nve.nevina) flows through Sandefjord Municipality and is Andebu's longest river, with a total length of 20 km (**Figure 5**) (Sandaas and Enerud, 2015). Skorgeelva drains from Lake Åletjørn (204 meters above sea level) and Lake Trollsvannet (174 meters above sea level) and receives inflow from, among others, Lake Langevann (elevation 174 meters above sea level) to the west, before it empties into Lake

Goksjø (28 meters above sea level) (https://www.kodal.info/index.php/River Skorgeelvaelva).

Despite being formally known as River Skorgeelva, it is referred to by several different names along its course. In the upper reaches, the merging of River Åletjønnselva and River Trollselva gives rise to River Hynne. As it flows from Trolldalen, it adopts the name River Nøklegårselva, while from Ådnesaga, it is referred to as River Slettingsdalelva. From Holmen, it is recognized as Skorgeelva until it takes on the name River Trollsåselva from Trollsås, ultimately reaching its destination in Lake Goksjø. In 1962, Goksjø was lowered, leading to the lowering and channelization of the lower reaches of Skorgeelva. Additionally, timber floating has historically been conducted in the watercourse, and at Bommerhus, booms were placed in the river to sort the timber (https://www.kodal.info/index.php/River Skorgeelvaelva; Larsen and Magerøy 2020).

River Skorgeelva´s catchment area covers an area of 60.1 km^{-2} (**Figure 6**), has a runoff of 20.4 $l/s*km^{-2}$, and is predominantly covered by forest (90%). Further south in the catchment area, the watercourse is somewhat affected by agriculture, runoff, and infrastructure. The river varies between areas with rapids and fast-flowing water, with a rocky riverbed, to pools and calm-flowing sections with sand and gravel (Sandaas and Enerud, 2015). The highest point in the catchment area is around 448 meters above sea level. During the summer the catchment area receives approximately 462 mm of precipitation, and during the winter about 600 mm (Norwegian Water Resources and Energy Directorate, nevina.nve.no).

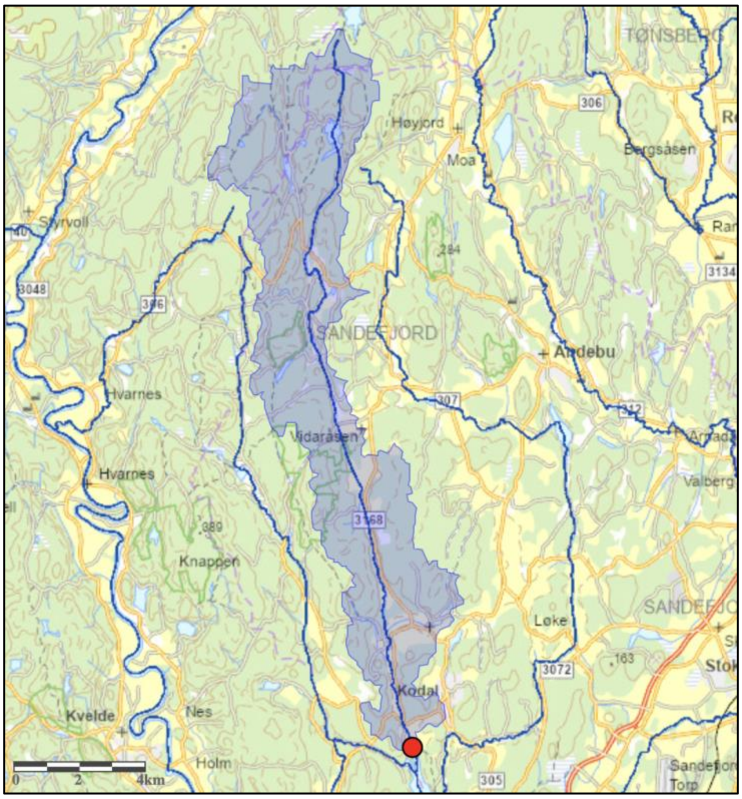

Figure 6 River Skorgeelva´s catchment area. Retrieved from the Norwegian Water Resources and Energy Directorate, nevina.nve.no.

Weather data was retrieved from seklima.met.no (https://seklima.met.no/observations/) for the period from May to October 2023. The data is from the Sandefjord area, which was the closest measuring station to River Skorgeelva. This area received a total of 559 mm of precipitation during the study period, with a peak in August (168,7 mm) due to the extreme weather event "Hans" (Granerød et al., 2023). The average air temperature was approximately 13°C, with a peak in June (17,6 °C) (**Figure 7**).

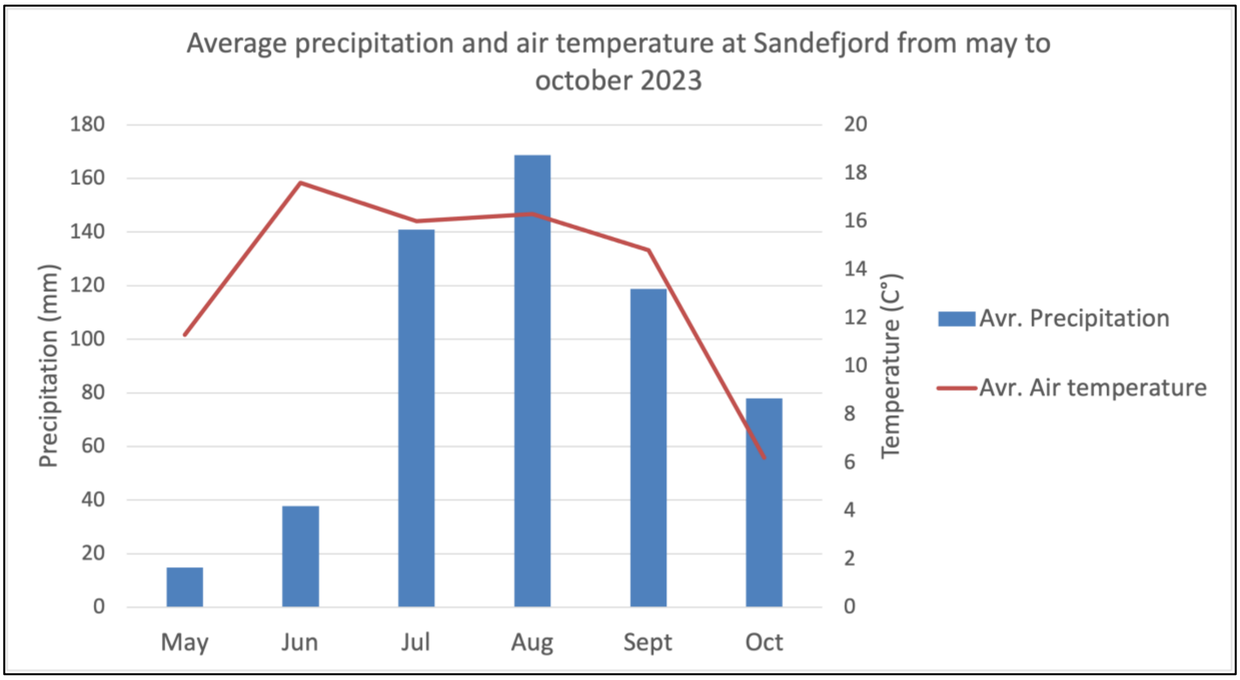

Figure 7 Temperature (°C) and precipitation (mm) measurements for the Sandefjord area during the study period. Retrieved from: https://seklima.met.no/observations/

2.1.2.2 Water quality in River Skorgeelva

In 1979-1981, water quality in River Skorgeelva was examined by Holtan and Brettum (1982). These investigations revealed a pH of 6.8, turbidity of 1.7 FTU and conductivity of 7.7 mS m^{-1}. The nitrate concentration varied from 150 to 1060 µg L^{-1}, while the concentration of calcium was 5.2 mg L^{-1}, and the concentration of iron was 240 µg L^{-1}.

Hansen (2005) conducted water quality sampling in River Skorgeelva in 2004 and found that the phosphorus concentration varied but increased downstream in the watercourse. At Storedalssaga, the concentration was 4.4 µg L^{-1}, while at Lake Ådnedammen and Slettingdalen, the average values were 12.4 and 10.8 µg L^{-1} respectively, indicating 'good' or 'very good' ecological status. In the lower part of the river, at Trollsås bridge, the phosphorus concentration was measured at an average of 16.9 µg L^{-1}, which indicates 'moderate' to 'good' ecological condition. It was also observed that the river experienced periods of increased phosphorus concentrations, for example during flood events in 2004. The highest values measured during this period were 8 µg L^{-1} (Storedalssaga), 20 µg L^{-1} (Lake Ådnedammen), 21 µg L^{-1} (Slettingdalen), and 36 µg L^{-1} (Trollsås bridge). This suggests that the river occasionally experiences 'moderate' or 'poor' ecological conditions for its river type. It is concluded that the occasionally high phosphorus concentrations are due to runoff from agriculture and sewage discharge.

Orthophosphate is the free phosphorus in water that is available for algal growth. A high proportion of orthophosphate typically indicates sewage water, while a lower proportion indicates surface runoff because a larger proportion is bound to particles. Water samples from the upper part of River Skorgeelva, at Storedalsaga, did not contain orthophosphate for much of the year. Greater variations were observed at Lake Ådnedammen and Slettingdalen, and at Trollsås bridge. Values up to 24 µg L^{-1} were measured (Hansen, 2005). This is mainly due to diffuse sewage, grazing animals, and in some areas, municipal sewage (Simonsen, 2008).

Water quality samples taken in River Skorgeelva by Larsen and Magerøy (2020) in 2019, showed turbidity measurements of <1 FTU. pH was measured at 6.9 in August 2019 and 7.8 in October 2019. The conductivity was moderate and somewhat increasing downstream in the river. Nitrate levels were measured at 35 µg L^{-1} in August and 190 µg L^{-1} in October. Total phosphorus was 5.1 µg L^{-1} in August and 7.5 µg L^{-1} in October. Regarding phosphorus and nitrogen measurements, Skorgeelva is therefore a river with close to 'very good' status, according to the Norwegian Water Framework Directive (2018). This suggests that the water quality in the river has improved over the past decades, although the sampling was very limited.

Water quality samples were not taken during our studies in River Skorgeelva. This was partly due to workload management and because samples had already been taken, in the period of 1979-2019, in the same areas (Hansen, 2005; Holtan and Brettum, 1982; Larsen and Magerøy, 2020).

In the context of monitoring freshwater pearl mussels in the river system in 2019 (Larsen and Magerøy, 2020), measurements of redox potential in the substrate (at a depth of 5-7 cm) were also taken. Measurements were conducted at two stations in August 2019 and at three stations in October 2019. The results of these measurements showed consistently lower redox potentials in the substrate in August (median values = 521, 462), but they remained satisfactory for freshwater pearl mussels as most measurements at both stations were >400 mV. In October, there were generally higher redox values in the substrate (median values = 515, 582, 565), with only one station recording a redox potential lower than 300 mV. This indicates that the conditions in the substrate are favorable for freshwater pearl mussel recruitment in large reaches of the river. Additionally, higher values of redox potential are important for the composition of the benthic invertebrate fauna (Knott et al., 2019).

2.1.2.3 Freshwater pearl mussel in River Skorgeelva

Freshwater pearl mussels have been recorded in River Skorgeelva since the late 19th century (Kleiven and Dolmen, 2012). In Dolmen and Kleiven (1997b)´s national overview of freshwater pearl mussel in Norway, Skorgeelva is mentioned as a site with a declining population since 1975. In 2009, mussels were recorded along a 15-kilometer reach, from Lake Lakstjernet to Lake Goksjø (Sandaas and Enerud, 2009). Based on previous surveys in the area, Skorgeelva was included in the national monitoring of freshwater pearl mussel in 2019, as a B-site (Larsen and Magerøy, 2020).

Gregersen (2018) calculated the population size of freshwater pearl mussels in River Skorgeelva to be around 87 000 (average river width of 2.5 m x mussel-bearing length of the river of 15 km), with a density of 2.31 individuals per m^{-2}. In 2019, new estimates by Larsen and Magerøy (2020) showed a significantly larger population size of 235 000 visible mussels, with a density of 6.28 per m^{-2}. This indicates a large and likely viable population. Additionally, it was estimated that a large number (20 000 individuals in 2019) were buried in the substrate. The highest densities in 2019 were found in the middle and lower reaches of the watercourse.

Sandaas and Enerud (2009) found a large and viable population in their studies. They observed good recruitment during their investigations of the watercourse. In 2019, there was an abundance of older individuals in the length group of 95-115 mm, with an average length of 95 mm. Mussels were found in all length groups larger than 20 mm, including ten individuals smaller than 50 mm, which accounted for 4.9% of the total number of inspected mussels. No individuals smaller than 20 mm were found, indicating inadequate recruitment (Larsen and Magerøy, 2020).

Although there was a predominance of salmon in River Skorgeelva, no mussel glochidia were found on any of the salmon fry examined in 2019. However, there was a high number on trout. The trout fry examined had a high number of glochidia, as well as a majority of individuals two years and younger being infected. This characterizes the mussel population in Skorgeelva as a "trout mussel" (Larsen and Magerøy, 2020).

2.1.2.4 Benthic macroinvertebrates and epiphytic algae in River Skorgeelva

In 2022, analyses of the benthic community in River Skorgeelva were conducted in connection with an assessment of the ecological status in several water bodies in the area (Norconsult AS, 2023). Samples were taken at a station near Trollsås bridge, from October 4th to November 1st during normal to high flow conditions (the exact number of samples taken is somewhat unclear). Benthic invertebrate samples were collected using the kick-sampling method (Direktoratsgruppen vanndirektivet, 2018). This method involves disturbing the riverbed ahead of a fine-mesh net, to collect benthic organisms carried by the water current.

The results of this survey revealed, among other findings, the presence of 13 families of EPT, including mayflies, stoneflies, and caddisflies. Six of these families were among the most pollution sensitive. Additionally, the sample was dominated by beetles (Hydrophilidae) and mayflies *(Caenis sp.).* An ASPT score of 6.43 was calculated for these samples, indicating a 'good' ecological status class (Norconsult AS, 2023).

Furthermore, earlier in the season (August 11th to September 1st), samples of epiphytic algae were taken at the same station during low flow conditions. Based on these samples, River Skorgeelva was classified as having a 'good' ecological status class. Consequently, the overall assessment reflects a 'good' ecological status (Norconsult AS, 2023).

2.1.2.5 Fish population in River Skorgeelva

When Lake Goksjø was lowered in 1962, an old dam was also removed, allowing salmon once again the opportunity to migrate up the River Skorgeelva to the River Slettingsdalselva (Gregersen and Thorsen, 2010; Larsen and Magerøy, 2020). However, there was a significant presence of pike in the watercourse, prompting several measures to eradicate them. For instance, the river was treated with rotenone between the Lakes Lakstjønn and Goksjø in 1962. Combined with pike barriers at Bjørndal and Slettingdalen, Skorgeelva was believed to be pike-free above Slettingdalen (Larsen, 1985; Larsen and Magerøy, 2020).

In 1985, electrofishing was conducted at a station near Trollsås, which revealed the presence of both salmon and trout in the watercourse, with a predominance of trout (75% of salmonids). Nine salmon fry and 26 trout fry were caught in a 50 m^{-2} area of the river (2 salmon fry (0+), 16 older salmon fry (≥1+), 46 older trout fry per 100 m^{-2}). The aim was to increase the number of salmon in the watercourse, so fingerlings were stocked until the mid-1970s (Larsen, 1985). Salmon can migrate up 22 km to the Lakes Trollsvatn and Åletjern (Larsen and Magerøy, 2020).

Larsen and Magerøy (2020) conducted a new electrofishing survey in River Skorgeelva in 2019, at three stations between Ådnesaga and Bjørndal. The results showed that salmon had become the dominant species. The density of salmon parr (0+) was 47 individuals per 100 m^{-2}, and for one-year-old or older salmon (≥1+) it was 16 individuals per 100 m^{-2}. Only sporadic findings of trout were made in the watercourse (density of 1.4 individuals (all age classes) per 100 m^{-2}). Thus, trout accounted for 2% of salmonids in Skorgeelva in 2019.

On Vann-nett.no (https://vann-nett.no/portal/#/waterbody/015-366-R), River Skorgeelva is classified as having poor ecological status due to the genetic effects of escaped farmed salmon. Therefore, Skorgeelva is included in the Norwegian Directorate of Fisheries' national monitoring program for escaped farmed salmon. Results from this monitoring program initiate actions to remove these fish.

2.2 Study design

We collected data on 4 occasions (June, July, September and October) from River Hoenselva and River Skorgeelva, during the summer of 2023. Each river contained three stations, selected to represent diversity in mussel densities and variation in hydromorphological conditions within the rivers. The stations were placed near previous surveys of river mussel populations, and they were strategically positioned to make it possible to take good benthic samples with a Surber sampler. Thus, we avoided stretches of the river that were dry, and we sought areas with some water movement to be able to take the benthic samples.

In River Hoenselva, we first chose four stations along the river, but due to workload limitations we had to omit one station. We chose to remove Station D, as the substrate was very uniform, with gravel and sand, and there were no apparent mussels at the surface when we were during our survey. Station A, B and C remained, and their location is shown in **Figure 8**, with UTM coordinates in **Table A 1.** In general, there was a lot of mixed forest observed around Station A and B (**Figure 9**). At these two stations, there were grazing animals with access to the river. The area around Station C was characterized by open cultivated land and forest. Water flow during the first field round helped determine the location of the stations, as we aimed to avoid dry areas and water that was too deep to stand in the river.

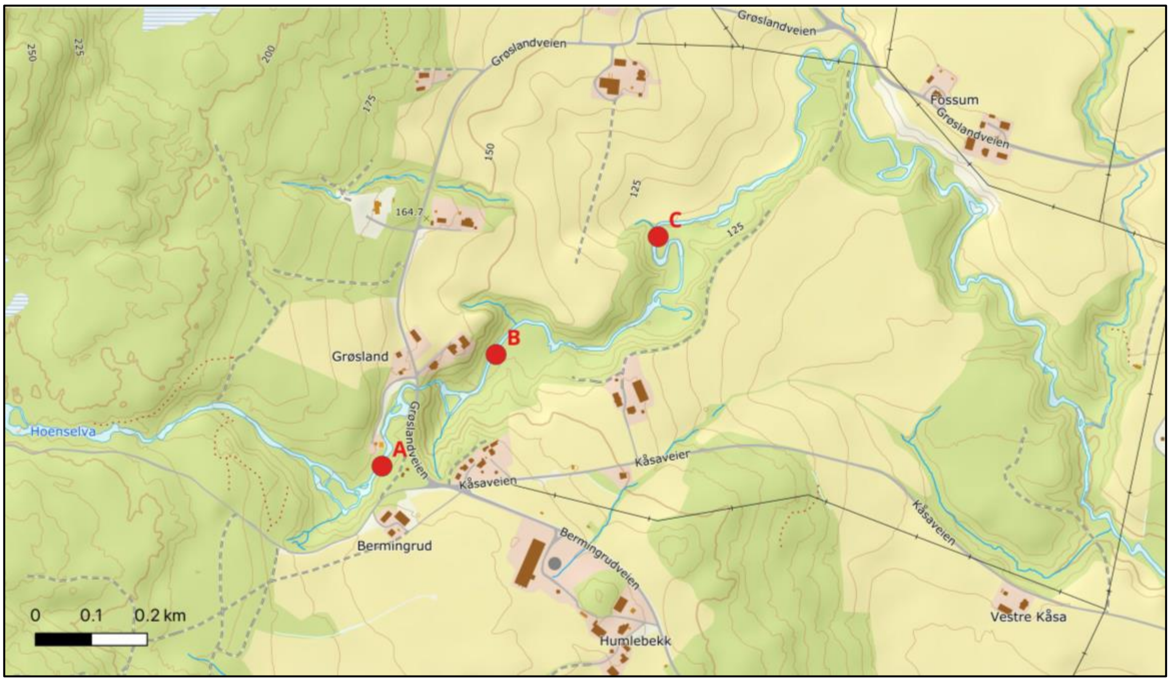

Figure 8 The stations utilized in River Hoenselva, Øvre Eiker municipality.

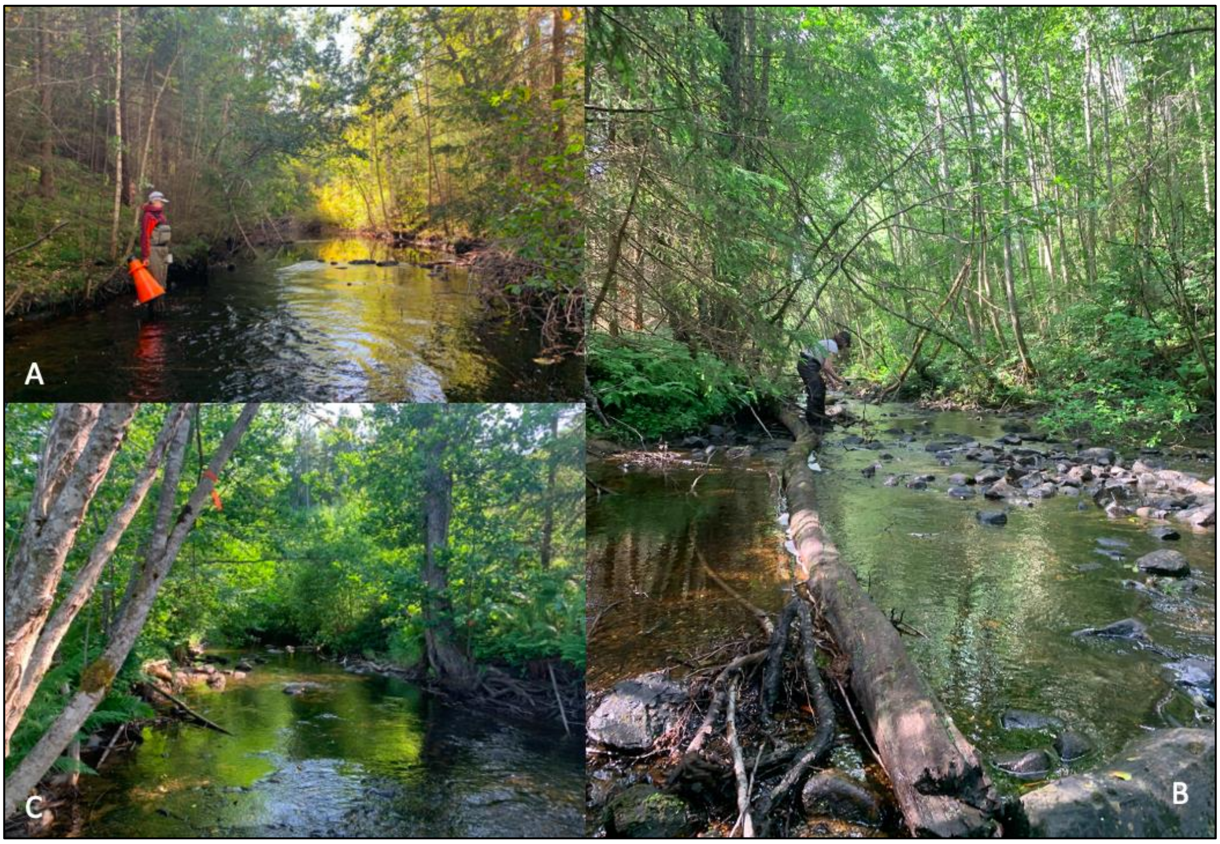

Figure 9 Photos of each station in River Hoenselva. Photos: Malin Nordstrøm Hansen, Mari Hildrum.

In River Skorgeelva, we also chose four stations, but due to workload limitations we had to omit one station. This time, we omitted Station B, as it was a bit too close to Station A, as well as there being few mussels apparent on the surface during the survey. Station A, C and D remained, and is shown in **Figure 10**, with UTM coordinates in **Table A 2.** The surrounding nature, near stations A and D, in Skorgeelva was characterized by mixed forest and some cultivated land (**Figure 11**). Station C was more influenced by open cultivated land, with little overhanging vegetation (**Figure 11**). Once again, water flow during the first field round helped determine the station locations, to avoid too little or too much water.

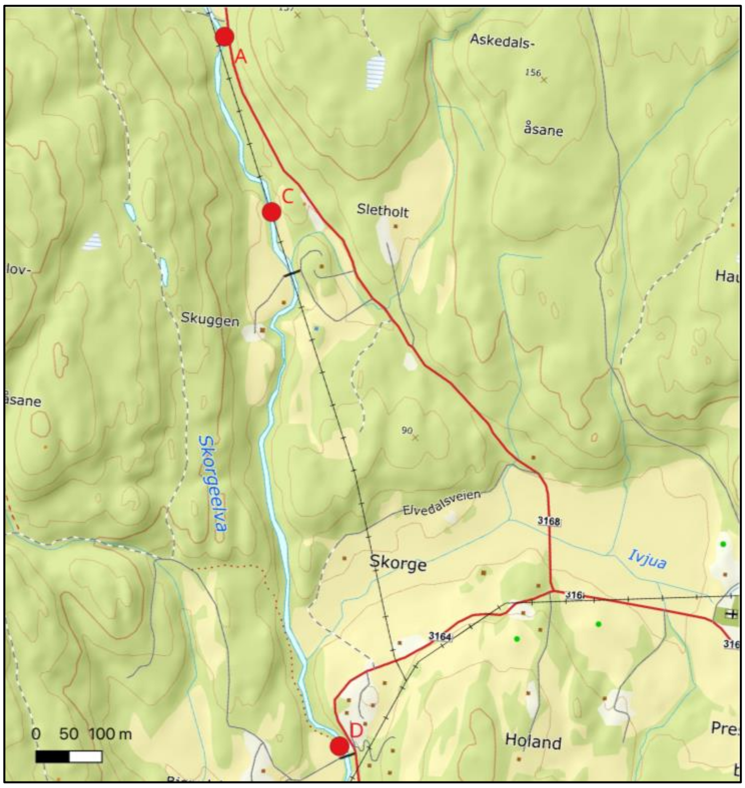

Figure 10 The Stations utilized at River Skorgeelva, Sandefjord municipality.

Figure 11 Photos of the stations at River Skorgeelva. Photos: Malin Nordstrøm Hansen, Mari Hildrum.

At each station, we laid out transects positioned perpendicular to the river's general direction of flow and strategically placed to cover the variability at the stations. It was also important for the water not to be too deep at the transects to allow for sampling, yet deep enough to avoid them likely drying up during the summer. They were placed with 1 meter or more between them, depending on the river channels (**Figure 12**). The length of the transects also varied a lot, based on the width of the river, river channels and dry riverbed (min. 2 cells, max. 9 cells).

Figure 12 Examples of transects. A: Example of a station with transects, River Skorgeelva Station D. B: One transect marked with rebars and chains pulled tight. River Skorgeelva, Station C. Photos: Malin Nordstrøm Hansen, Mari Hildrum.

To lay out each transect, we used 50 cm long rebars that we hammered down into the substrate in each corner of the transect. We then pulled chains tight across the rebars, both at the top and the bottom, with cable ties marking exactly 50 cm in length (**Figure 12**). That way, we had precise markings for where our cells were laid out, with the same size of 50x50 cm. We also had a smaller chain that we could move between transects, to mark the boarders between the cells in the same transects. Using pictures, bands in the surrounding vegetation and noting down distance to the riverbank and the other transects, the transects were laid out in the same spot each field round. The number of transects varied from 7-13 based on the station, but in total we had 45 cells (50x50 cm) that were examined at each station.

We then made different categories of data collections, which were assigned to each cell by doing a randomization in Microsoft Excel (Microsoft Corporation, 2023). The only data collection done every time and in every cell is the counting of freshwater pearl mussels on the surface. The rest of the data collections were split into 6 categories. **Table 1** is an example of one of such randomizations done for this study, from River Hoenselva station A.

Table 1 Randomization of River Hoenselva Station A. The first columns give information on station and transects, showing where in the river we are. The middle portion of the table shows what kind of sampling was undertaken in the different cells. "H" is hydromorphological measurements, "G" is digging for freshwater pearl mussels (*Margaritifera margaritifera*), "S6" is Surber cells in June, "S7" is Surber cells in July, "S10" is Surber cells in October. "R" is reserve cells. Selected cells are the total number of cells that were used for the data collection.

		Position in the river (left to right looking up the stream)							
Station	**Transect**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Selected cells**
A	1	S6	H	G	R	S10	R	G	7
A	2	R	H	G	S7	R			5
A	3	S10	H	G	R	S6			5
A	4	S10	G	R	H	S7	G		6
A	5	H	S6						2
A	6	G	H	R	S7				4
A	7	R	S10	G	S6				4
A	8	S7	H	R					3
A	9	H	G						2
A	10	H	S6						2
A	11	S7	H	G	R	S10			5

The first category includes hydromorphological ("H") data collection, which consists of measuring the waters velocity, depth, quantifying the substrate composition, as well as the redox potential. The hydromorphological data was sampled in 10 cells at each station.

Three of the categories are related to the benthic invertebrate fauna sampling and were spread over three different months. There was one in early summer, labelled "S6", which was executed in June. One in summer, labelled "S7" which were executed in July, and another one in autumn, executed in October labelled "S10". The categories consist of 5 cells at each station. In these cells, hydromorphological sampling was also performed.

The next category is digging for freshwater pearl mussels, which is applied to 10 cells and labelled "G". This fieldwork was only executed once during our project and was undertaken during the start of September. Hydromorphological analyses were also performed in these cells.

The last category is the reserve cells labelled “R”, consisting of 10 cells in each station. These were used if the original cells were unavailable for sampling, for instance if one of the cells have dried up during summer, too deep for our tests to be taken correctly if a large rock hinders the use of the Surber-sampler. We utilized 14 reserve cells in total, as seen in **Table A 5** in appendix.

Due to an error, only 43 surveyed plots were examined in the River Skorgeelva at Station D. In the randomization, there was a missing “hymo cell' and a “reserve cell”. This was not discovered until the final field round, and therefore some hydromorphological data and mussel counts are missing at this station.

2.3 Fieldwork

2.3.1 Riverbed substrate analysis

We followed the Norwegian standard for describing the substrate composition, called NS-EN ISO 14688:2018 (Standard Norge, 2018). This standard describes the substrate compositions as shown in **Table 2**. We also added a category for plant cover, as well as a category for the Freshwater Pearl Mussels itself.

Table 2 The substrate types over the riverbed habitats for freshwater pearl mussels (*Margaritifera margaritifera*). From NS-EN ISO 14688-1:2018, with two additional categories, number 0 for the plant cover and 11 for the freshwater pearl mussel.

No.	Substrate type	Substrate size (mm)	No.	Substrate type	Size (mm)
0	Plant cover	Any plants	**6**	Gravel (Coarse)	>20-63
1	Roots/sticks/other	Anything	**7**	Cobble	>63-200
2	Silt	>0.002-0.063	**8**	Boulder	>200-630
3	Sand	>0.063-2.0	**9**	Large Boulder	>630
4	Gravel (fine)	>2.0-6.3	**10**	Bare bedrock	Any exposed bedrock
5	Gravel (medium)	>6.3-20	**11**	Freshwater Pearl Mussel	Any freshwater pearl mussels in the surface

Using this standard, we calculated a percentage of each of the substrate types in the chosen cell. The percentages where then used to make a substrate index, following the methods of Wacker et al., (2020) and Hedger et al., (2005). Firstly, the substrate types were evaluated to being available or not to the benthic invertebrate fauna. Seeing as benthic macroinvertebrates can both be buried into the substrate and cling to larger rocks, all substrate types were evaluated as available. Each substrate type was classified according to size and given a value in an ascending order. "Class 1" (roots/sticks/other) were given value 1, "class 2" (silt) was given value 2 and so on. These values were multiplied with the percentage from the same class. An example can be a cell where there were 20% fine gravel (4), 30% medium gravel (5), 20% coarse gravel (6) and 30% cobbles (7); the equation was $0{,}2*4 + 0{,}3*5 + 0{,}2*6 + 0{,}3*7 = 5{,}6$. This way the index gives us a scale of the substrates size.

2.3.2 Water velocity and depth

The water velocity was measured using an Advanced Stream Flowmeter from Geopacks, model MFP126-S. The flow meter was held at approximately 60% of the depth, estimated from the water surface down to the substrate. 60% depth were used because the velocity here is assumed to be equal to the mean velocity in the water column (Carter and Anderson, 1963). An estimation was made if the waterflow were to slow for the flowmeter to register any movement. The depth was calculated based on the average of four to five measurements in the cell. The measurements were performed with a folding rule.

2.3.3 Redox potential

The redox potential (E_H) is a measure of the reducing or oxidizing capacity of water (Økland and Økland, 1998), and signifies the water's capacity to both release and accept electrons. Environments rich in oxygen typically exhibit a higher redox potential owing to their increased ability to accept electrons. Conversely, environments characterized by lower oxygen levels demonstrate a diminished redox potential, due to their restricted ability to accept electrons (Schlesinger and Bernhardt, 2013).

When fine-grained material settles on the riverbed, it can clog the interstitial spaces in the sediment and reduce the exchange of water from the free-flowing masses (Arntzen et al., 2006; Rehg et al., 2005), consequently decreasing the amount of oxygen in the substrate (Denic and Geist, 2015) and reduce the habitat quality for many river-dwelling macroinvertebrates (Geist and Auerswald, 2007).

Strayer et al., (1997) demonstrate that the amount of dissolved oxygen in the substrate can be crucial for the density of macroinvertebrates in rivers. In addition, the composition of the benthic community has been shown to be affected by redox potential in interstitial zones (Knott et al., 2019). Moreover, there are several examples showing that oxygen-rich substrate (6.9 mg L^{-1} dissolved oxygen), with higher redox values (over 400 mV), is necessary for the development of both eggs and larvae of salmonid fish (Ingendahl, 2001), and for the survival of juvenile mussels (Geist and Auerswald, 2007; Gosselin, 2015; Stoeckl et al., 2020). This has proven to be a highly limiting factor for freshwater pearl mussel populations, as recruitment is negatively impacted by low oxygen levels in the substrate. Small mussels live buried in the substrate and depend on filtering oxygen-rich water in the interstitial areas (Larsen et al., 2012).

To assess the suitability of the riverbed as a habitat for mussels and invertebrates, we therefore utilize the redox potential of the substrate to evaluate the oxygen content in the interstitial spaces, following the methods outlined in (Geist and Auerswald, 2007). The method has been further developed and is used in several European countries (Larsen et al., 2012). The equipment used consisted of a measuring device provided by Dr. Frank Krüger at ELANA Boden Wasser Monitoring, featuring a 1.5 m long probe with a platinum electrode at one end, a reference electrode, and a voltmeter to record the measurements. The measurements were conducted at all stations during all four field campaigns (June, July, September, and October). At each station, 14-16 measurements were taken in the substrate and 7-8 measurements in the free-flowing water within selected (randomized) cells (Magerøy, 2023). In addition, we conducted field-surveys aimed to investigate the recruitment of freshwater pearl mussel through a “digging-survey” in September. This survey included 20 measurements of redox potential in the interstitial spaces and 10 measurements in the water column.

When measuring the redox potential in the free-flowing water, both electrodes were held in the upper water layer. When measuring the substrate, the reference electrode was held in the upper water layer while the platinum electrode was inserted approximately 5 cm into the substrate. To ensure accurate measurements, the equipment usually needed some time to stabilize. Previously, a standard of 3 minutes has been used to achieve this (Larsen et al., 2012). However, experience with the method has shown that the equipment may not necessarily require that much time if the values stabilize after 1-2 minutes. Therefore, the measurements were recorded when the values had stabilized after 1-3 minutes. If it was not possible to measure the redox potential in the substrate due to difficulties inserting the platinum electrode into the substrate (e.g., due to large stones or clay), the measurements were taken in the immediate vicinity of the original measurement point (Magerøy, 2023).

2.3.4 Freshwater pearl mussels

Living and dead mussels on the surface were counted using a water scope (bathyscope). In addition, specific 'dig cells' were selected within several of the transects. Here, visible (living) mussels were picked up. The cell was then further examined by removing rocks and lightly digging into the substrate to uncover any buried mussels. All mussels found in each route were measured to the nearest 1 millimeter using a caliper and returned to the same location on the riverbed (Wacker et al., 2020).

2.3.5 Surber sampling

Quantitative benthic invertebrate samples were collected using a Surber sampler, with a frame opening of 30x30 cm (0.09 m^{-2}) and a mesh size of 250 µm (Surber, 1969, 1937) The collection of Surber samples involved pressing the Surber net frame into the substrate within a selected cell. This was done to prevent gaps between the frame and the substrate, where benthic organisms could escape. We agitated the substrate within the frame using our hands for approximately one minute. Occasionally it was necessary to create water flow with one of our hands if the water velocity was low. Larger rocks were picked up and scrubbed, making the attached benthic macroinvertebrates flow into the net. If there were any mussels in the cell, we picked them up after the frame was placed into the substrate, moving them into a bucket where they were gently scrubbed. After, they were placed into another bucket, while the remaining "dirty" water was poured into the Surber net, making sure any benthic macroinvertebrates on the mussels were included in the sample. After collection, the entire sample was transferred to a sample bottle and preserved in 70% ethanol until further processing in the laboratory. Mussel was returned to their previous location.

2.4 Benthic fauna analysis

To analyze the benthic invertebrate community, we extracted the macroinvertebrates, i.e. benthic fauna visible to the naked eye (Jacobsen et al., 2008), separated them from the other organic and inorganic material in the sample and placed them on glasses. EPT taxa was separated from the rest of the sample and placed on separate glasses for analysis by NINA (Norwegian Institute for Nature Research) experts. We were trained by NINA in methods for determining the rest of the benthic animals using keys they had brought (Hubendick, 1949; Merritt et al., 2008; Nilsson, 1990, 1983, "Trollsländor: Nyckel till larverna," Unpublished). We used stereoscopic microscopes from Zeiss, model Stemi 305, with a magnification of 8x-40x. We wanted to identify benthic fauna down to the lowest possible taxonomic level, preferably to species.

Using Excel (Microsoft Corporation, 2023), we calculated the number of invertebrates, the number of invertebrate species and density of macroinvertebrates at each station. In addition, the number of EPT-invertebrates (individuals within EPT taxa), the number of EPT-species and density of EPT-taxa at each station was calculated. Lastly, we calculated the number of live and diseased freshwater pearl mussel, as well as density within the cell and within the Surber frame.

ASPT and RAMI were calculated by NINA experts. For Evenness, Shannon-Wiener-Index and filter feeders (active and passive), the online calculation tool Perlodes was used (https://gewaesser-bewertung-berechnung.de/index.php/home.html). The ecological status was determined according to the classification guidelines outlined in the Norwegian Water Framework Directive (2018).

2.5 Statistical analysis

We used both Microsoft Excel and RStudio (Posit team, 2023) for datamining and the statistical analyses. The data was tested for normality with Shapiro Wilk test. Data not normally distributed was tested for differences with Mann Whitney U test (two groups) and Kruskal Wallis test (more than two groups). For normally distributed data, differences were tested with t-tests. A p-value of less than 0.05 was considered to indicate statistical significance.

To explore and visualize the composition and similarities of benthic communities, we started with a Nonmetric Multidimensional Scaling (NMDS) (Shafii et al., 2013). Because of the sampling methodology (Surber), we chose to merge the benthic animals into one at the same station. This is because the area inside the Surber frame is small (0.09 m^{-2}), and we wanted to get a better picture of the benthic community at the station by looking at a larger area. By aggregating the data, we might lose connections in the data. However, then we might get a more precise picture of the entire benthic invertebrate fauna, as the fauna can vary a lot over small areas. Hence, the data used for the NMDS were station-based data.

To perform an NMDS, we used the package 'Vegan', and the meta_NMDS function with Bray Curtis dissimilarity. Then, we used 'ordiplot' and 'orditorp' to plot the NMDS-scores. After visualizing, we used the package 'ggplot' to further plot the NMDS-scores with symbols for the months and stations, and different colors for the river using the 'hull' function. An ANOSIM-analysis were used to calculate difference between rivers, months, and stations. Further, we used a SIMPER-analysis to find out which species contributes the most to the differences, still using a Bray Curtis dissimilarity. Lastly, we used the function 'envfit' to find out which of our environmental variables were significant. After scaling down to only the significant variables, we add these to our ordination plot (**Figure 16**). Based on the ordination plot we decided on which variables to use for further analysis.

To investigate relationships between our variables, we created a correlation matrix using Spearman rank correlation. The correlation matrix was generated using the 'corrplot' package in RStudio. To visualize significant p – values of the correlations, we used the 'Hmisc' package.

In our plots, we chose to display correlations with p-values less than 0.05. We chose to look at correlations between different densities of the benthic macroinvertebrates, density of the freshwater pearl mussel, benthic macroinvertebrate indexes and the different hydromorphological variables.

To further test our data, we intended to execute Generalized linear mixed models (GLMMs). This model is chosen because it allows the response variables to be random, and for data to be clustered (Rabe-Hesketh and Skrondal, 2010). Based on the ANOSIM executed in our ordination, we saw that river need to be random, with station nested into river. However, we also tried models without the different nesting and randomizations. To perform a GLMM, the 'glmer' function from the package 'lme4' is used, with Poisson distribution.
The GLMMs we intended to test had the following form:

```
glmer_model <- glmer (Response variable ~ Predictor variable1 +
                      Predictor variable2 + Predictor variable3 +
        random variables + (1|river) + (1|Station: River) + 1|Season),
    data = Station_data, family = Poisson)
```

The GLMM testing did not proceed as expected. When attempting to conduct the tests, we encountered issues with the models. We found that we have insufficient data to execute the models we intended to perform. We tried with both the single-cell dataset and the aggregated station-based dataset, but none of them were extensive enough. Thus, we had to base our analyses on the correlations and NMDS modelling.

3 Results

3.1 Hydromorphological parameters

Table 3 Water temperature (°C) at each station in River Hoenselva and in River Skorgeelva, by month.

Month	River Hoenselva			River Skorgeelva		
	Station A	Station B	Station C	Station A	Station C	Station D
June	18	18	17	21	21	22
July	18	18	17	16	17	16
September	16	14	12	16	15	15
October	7	9	8	9	7	6

Mean water temperature in River Hoenselva was 14.3°C and mean in River Skorgeelva was 15. 1°C. There was no statistically significant difference in water temperature between the two rivers (Wilcoxon test, W = 24040, p-value = 0.7851). However, the temperature differed significantly between months (Kruskal-Wallis, χ^2 (3) = 342, p-value = 8.53e-74). The highest temperature was measured in the summer months, June (mean = 19.5°C) and July (mean 17°C). September (mean = 14.6°C) and October (mean = 7.6°C) measured somewhat lower water temperatures (**Table 3**).

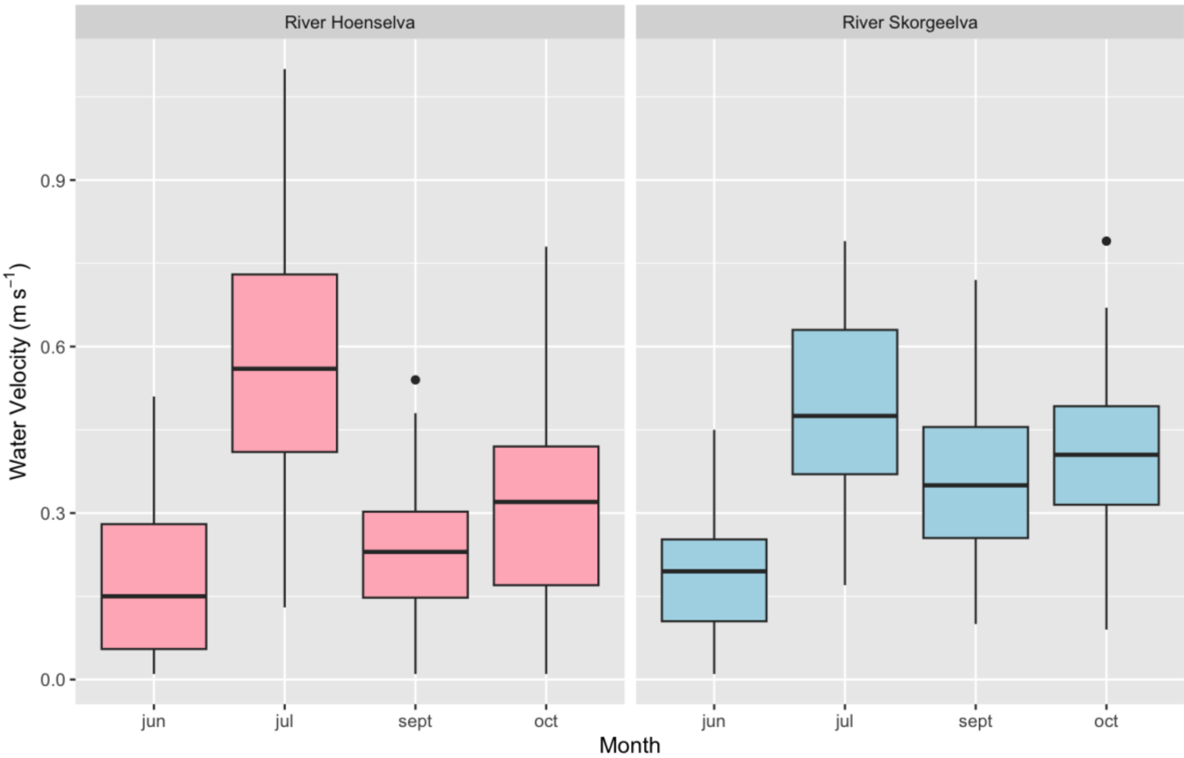

Figure 13 Monthly water velocity (m s^{-1}) in River Hoenselva and in River Skorgeelva.

In River Hoenselva, mean velocity was 0.31 m s^{-1}, and in River Skorgeelva mean velocity was 0.36 m s^{-1}. There was a significant difference in water velocity between the rivers (Wilcoxon test, W = 22288, p-value = 0.001654). Also, there was a difference in velocity per month (Kruskal-Wallis, χ^2 (3) = 145, p-value = 3.39e-31). The highest velocity was measured in July in both rivers (Hoenselva mean in July = 0.59 m s^{-1}, Skorgeelva mean in July = 0.49 m s^{-1}). This is due to the dry period in June and the heavy rainfall in July (**Figure 13**).

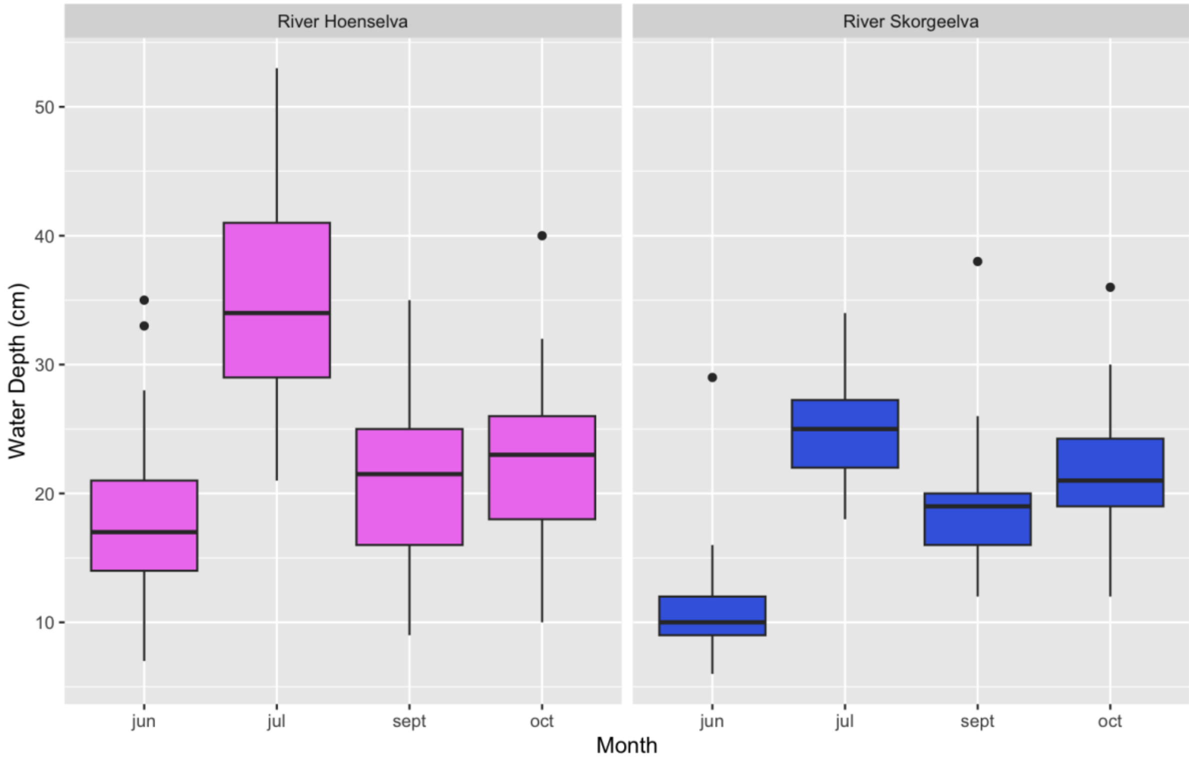

Figure 14 Monthly water depth (cm) in River Hoenselva and in River Skorgeelva.

Mean water depth in River Hoenselva was 23.6 cm, and mean water depth in River Skorgeelva was 19 cm. There was a significant difference in water depth between the rivers (Wilcoxon test, W = 24040, p-value = 2.182e-06). Also, there was a difference in depth per month (Kruskal-Wallis, χ^2 (3) = 174, p-value = 1.54e-37). The largest depth was measured in July in both rivers (Hoenselva mean in July = 34 cm, Skorgeelva mean in July = 24 cm). The smallest depth was measured in June in both rivers (Hoenselva mean in June = 17 cm, Skorgeelva mean in June = 10 cm) (**Figure 14**). This might be because of little precipitation in June and a lot of precipitation in July (**Figure 4; 7**).

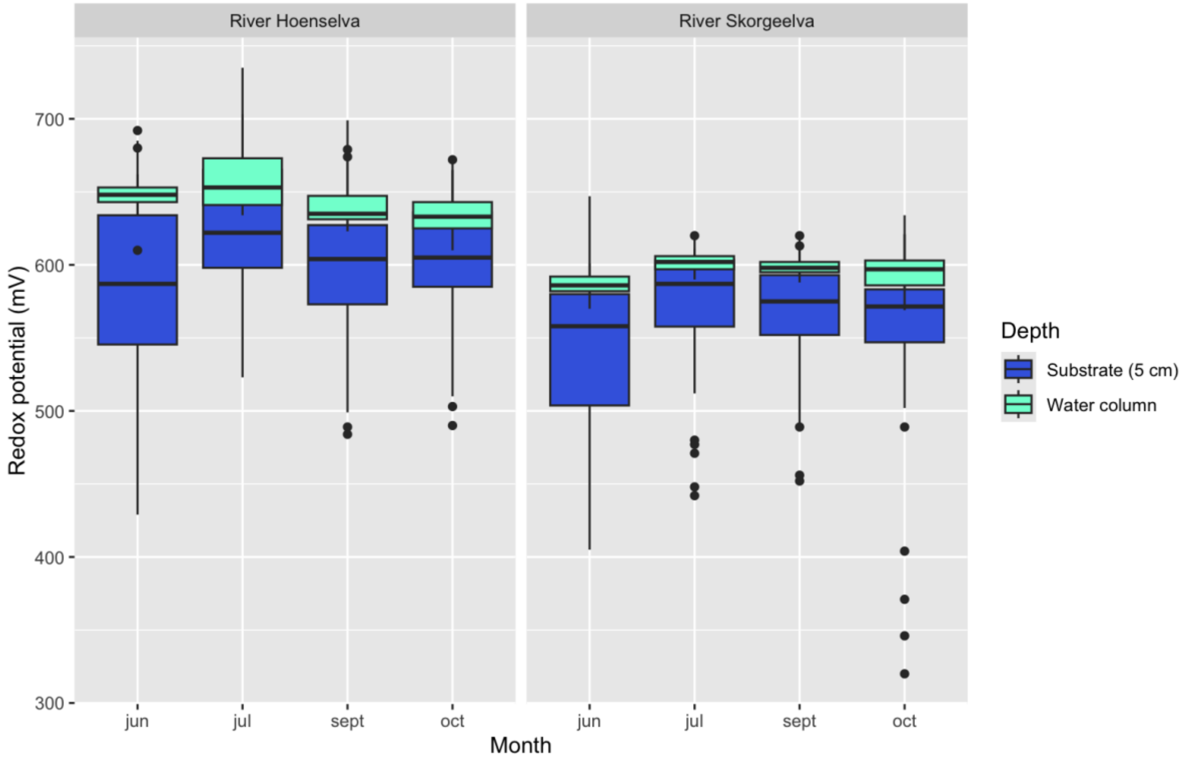

Figure 15 Monthly redox potential E_H (mV) in River Hoenselva and in River Skorgeelva, in the water column and the substrate (5 cm deep).

We measured the redox potential in both the water column and in the substrate. As measurements in the substrate is stated to be sufficient to evaluate microhabitats (Geist and Auerswald, 2007; Knott et al., 2019), we will continue with only the measurements in the substrate.

There was a significant difference in redox potential in the substrate between River Hoenselva and River Skorgeelva (Wilcoxon test, W = 28362, p-value = 2.2e-16). Median of redox potential in Hoenselva was 609 mV, and in Skorgeelva it was 573 mV. There was also a significant difference by month (Kruskal-Wallis χ^2 (3) = 20.3, p-value = 0.000145). The highest measurements were taken in July, while the lowest measurements were taken in June, in both rivers. All measurements in the substrate were above 400 mV (**Figure 15**).

Table 4 Substrate index across all stations in River Hoenselva and River Skorgeelva.

	River Hoenselva		River Skorgeelva	
Station	A	6.75	A	6.64
	B	6.76	C	6.45
	C	6.44	D	6.74

The substrate index for each station is shown in **Table 4**. Substrate index in River Hoenselva was 6.62 and in River Skorgeelva it was 6.67. There was no statistically significant difference in substrate index between the two rivers (Wilcoxon test, W = 18810, p-value = 0.9978). Generally, the riverbed was dominated by gravel and cobble, in both rivers.

3.2 Freshwater pearl mussels

Table 5 Density of freshwater pearl mussels (*Margaritifera margaritifera*) (individuals m^{-2}), with surface counting in River Hoenselva, across stations.

River Hoenselva	Station A	Station B	Station C
June	15.82	2.49	3.02
July	16.0	2.22	2.76
September	14.49	2.22	2.40
October	13.69	1.60	2.22

Overall, we found on average 222 living mussels in River Hoenselva. The densities are shown in **Table 5**. In Hoenselva, the densities were larger at station A, which was the furthest upstream of our stations (**Figure 2**). The results from the freshwater pearl mussel digging show that the recruitment for our stations were quite poor with only 10 juvenile mussels under 50 mm (17%) and 4 mussels under 20 mm (**Table 6**). However, these findings indicate 'very good' ecological condition based the Norwegian Water Framework Directive (2018). We found the most juvenile mussels at Station A.

Table 6 Results from the freshwater pearl mussel (*Margaritifera margaritifera*) digging in River Hoenselva, across stations.

Station	Location	n of mussels in total	n of mussels above 50 mm	n of mussels below 50 - 21 mm	n of mussels below 20 mm	Max. of length (mm)	Min. of length (mm)	Average length (mm)
A	Surface	35	34	1	0	109	49	82.5
	Substrate	15	8	4	3	92	6	44.5
B	Surface	1	0	1	0	77	77	77
	Substrate	2	1	0	1	55	18	36.5
C	Surface	5	5	0	0	96	70	82
	Substrate	0	0	0	0	-	-	-

Table 7 Densities of freshwater pearl mussels (*Margaritifera margaritifera*) (individuals m^{-2}), with surface counting in River Skorgeelva, across stations.

River Skorgeelva	Station A	Station C	Station D
June	17.96	14.13	1.21
July	22.76	16.09	1.77
September	14.93	14.22	1.95
October	17.96	13.33	1.95

Overall, we found on average 388 living mussels in River Skorgeelva. Densities was highest at Station A and C, and the station with the lowest density was Station D (**Table 7**). In Skorgeelva, the result from the freshwater pearl mussel digging is shown in **Table 8**. Here, we see that the recruitment is quite poor, with only 12 juvenile mussels under 50 mm (7%), and 1 mussel under 20mm. This indicates 'good' ecological condition based the Norwegian Water Framework Directive (2018). Generally, there was few mussels found at Station D, with only 8 mussels recorded in the digging cells.

Table 8 Results from the freshwater pearl mussel (*Margaritifera margaritifera*) digging in River Skorgeelva, across stations.

Station	Location	n of mussels in total	n of mussels above 50 mm	n of mussels below 50-21 mm	n of mussels below 20 mm	Max. of length (mm)	Min. of length (mm)	Average length (mm)
A	Surface	87	86	1	0	136	32	93.6
	Substrate	14	6	8	0	92	25	53.9
C	Surface	60	60	0	0	127	58	107.6
	Substrate	4	2	2	0	75	24	47.3
D	Surface	6	6	0	0	101	70	86.5
	Substrate	2	1	0	1	73	14	43.5

These results show that there still was recruitment in the rivers, but it is insufficient and varying. Due to limited results, we chose to shift the focus of the study away from recruitment and towards benthic invertebrates and their community composition in areas where mussels are present.

3.3 Benthic macroinvertebrates

We found a total of 45 653 individuals, divided by 94 taxa. 60.78%. of all individuals were found in River Skorgeelva, and 39.22% were found in River Hoenselva. The most abundant taxa in both rivers were Chironomidae larvae (42% of total) and *Baetis rhodani* (16% of total). 70% of Chironomid larvae were found in the June samples. Simultaneously, 70% of Baetis rhodani were found in the October samples (**Table A 7**). 72.3% of the total number of species was EPT-species.

Table 9 An overview of the number of species and individuals of benthic macroinvertebrates (excluding the freshwater pearl mussel (*Margaritifera margaritifera*)), number of EPT taxa and number of individuals, both river-wise and station-wise.

	Station	Number of Benthic macroinvertebrates species	Number of Benthic macroinvertebrate individuals	Number of EPT species	Number of EPT individuals
River Hoenselva	A	56	7464	39	1805
	B	58	4408	40	1992
	C	57	6031	38	2841
	Total	**77**	**17 903**	**54**	**6638**
River Skorgeelva	A	64	8771	44	2935
	C	58	8445	37	3178
	D	65	10 529	44	4027
	Total	**79**	**27 745**	**55**	**10 140**

In River Hoenselva, we found 77 taxa and 17 903 individuals. 54 taxa were EPT species (70.1%) (**Table 9**). In River Skorgeelva, we found 79 taxa and 27 745 individuals. This is almost 10 000 more individuals than in the Hoenselva (**Table 9**). Of the 79 taxa, 55 were EPT species (69.6%). There was a similar abundance of EPT species in the two rivers. Overall, the number of species was quite similar, but the number of individuals was larger in Skorgeelva. The samples were taken on equal total area in both rivers.

In River Hoenselva, 12 taxa were Ephemeroptera (E), 15 taxa were Plecoptera (P), and 27 taxa were Trichoptera (T) (**Table A 4**). Therefore, Trichoptera was the richest group of EPT in this river. In River Skorgeelva, 13 taxa were Ephemeroptera (E), 18 taxa were Plecoptera (P), and 24 taxa were Trichoptera (T) (**Table A 4**). Also in this river, Trichoptera was the richest EPT group.

In terms of benthic macroinvertebrate diversity, we observed a significant difference in the Shannon index (**Table A 3**) between the rivers (Wilcoxon test, W = 17, p-value = 0.03998), indicating higher diversity in River Hoenselva (0.50 - 2.58) compared to River Skorgeelva (0.73 - 2.45). A significant difference was also recorded between the months (Kruskal-Wallis, χ^2 (2) = 23.6, p- value = 7.49e-06), with June being the month with the lowest species diversity (**Figure A 1**). Additionally, species were more evenly distributed in Hoenselva (0.18 – 0.87) than in Skorgeelva (0.27 – 0.74), as indicated by the Evenness index (Wilcoxon test, W = 1513.5, p-value = 5.369e-05) (**Table A 3**).

No difference was detected between the rivers' degree of influence of eutrophication (ASPT) (t-test, t = -0.15508, df = 87.257, p-value = 0.8771). ASPT in River Hoenselva was 4.85 – 6.90, and in River Skorgeelva it was 5.25 – 7.05 (**Table A 3**). Based on the Water Framework Directive's classification guide, this indicates 'good' ecological condition in both rivers regarding eutrophication (Direktoratsgruppen vanndirektivet, 2018).

When considering the acidification index RAMI, a significant difference was observed between the rivers (t-test, t = -3.8294, df = 87.762, p-value = 0.0002405), although both rivers exhibited high index values (**Table A 3**). RAMI in River Hoenselva was 5.05 – 6.19, while in River Skorgeelva it was 5.14 – 6.45. Consequently, both rivers was classified as having a 'very good' ecological status concerning acidification (Direktoratsgruppen vanndirektivet, 2018).

Table 10 The density (individuals m^{-2}) of benthic macroinvertebrate fauna (not included the freshwater pearl mussels) and the EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa in River Hoenselva and River Skorgeelva, both river-wise and station-wise.

	Station	Benthic macroinvertebrates (individuals m^{-2})	EPT taxa (individuals m^{-2})
River Hoenselva	A	5529	1337
	B	3265	1476

	C	4467	2104
	Total River	**4421**	**1639**
River Skorgeelva	A	6497	2174
	C	6256	2354
	D	7799	2983
	Total River	**6851**	**2504**

The density of benthic macroinvertebrates in Surber samples is presented in **Table 10**. Overall, the total density in the rivers indicates that River Skorgeelva generally had higher densities of both benthic macroinvertebrates and EPT species.

We found a varying proportion of filter feeding (active and passive) macroinvertebrates in our samples (0.1 – 24.6%) (**Table A 6**). On average, there were 15% filter feeders in River Hoenselva and 10% in River Skorgeelva. Thus, there was also a significant difference between the rivers (Wilcoxon test, W = 678, p-value = 0.006618).

Two benthic samples from cell S07C9-3 and H07A8-1 were damaged during transport and were dry when they were to be analyzed. This does not appear to have destroyed the samples, and a similar number of species compared to the other samples were recorded, in addition, the indexes did not appear to be affected by this.

3.4 Statistical analysis

3.4.1 NMDS modeling

A non-metric multidimensional scaling (NMDS) analysis was used to investigate how the benthic invertebrate community was related to the hydromorphology and the mussels. The model shows how the abundance of different benthic macroinvertebrate communities (in our case, each individual cell is meant to be an individual community) will be organized when made into two dimensions. This helps visualize how the different communities are grouped in relation to each other, and to the environmental variables affecting the community (Dexter et al., 2018). In **Figure 16,** we see that the benthic macroinvertebrate fauna arranges itself into groups. This is further proven by the ANOSIM test, which shows that the "months" are a significant grouping (ANOSIM R = 0.5807, p- value = 1e-04). The different rivers were also shown to be grouped together, but when using the Station data, “river” is only close to being a significant grouping (ANOSIM R = 0.1612, p-value = 0.0608). To find out which one of our environmental variables were significantly affecting the benthic invertebrate fauna, an Envfit test were performed. The significant variables were, the temperature (Envfit test $r^2 = 0.7451$, p-value = 0.001), redox potential in the substrate (Envfit test $r^2 = 0.5301$, p-value = 0.007) and the substrate index (Envfit test $r^2 = 0.4760$, p-value = 0.013).

Temperature significantly affects the composition of the benthic macroinvertebrate fauna. Temperature lies on a horizontal axis in **Figure 16,** indicating that it has a similar impact on both rivers. This was also demonstrated in Chapter 3.1, where it was shown that there was no significant difference in temperature between the two rivers. We observe that temperature also varied between months, as depicted in **Figure 16,** where the stations was grouped monthly along the same axis, with June closest to the temperature axis.

Substrate index is on the same axis as temperature. We also observe that the substrate index is not significantly different between the two rivers. Since it lies on the same axis as temperature, we can see that it also varies between months, as they cluster with October closest to the substrate index axis. This indicates that substrate coarseness has a different influence for the benthic macroinvertebrate community at different points in time.

In **Figure 16,** the redox potential in the substrate lies on a distinct axis, indicating its independence from the other hydromorphological parameters measured. This suggests that redox potential contributed uniquely to the structure of the benthic macroinvertebrate community. We also observe that it lies vertically across the rivers, indicating a significant difference between the two rivers. **Figure 16** shows that River Skorgeelva is closest to the axis for redox potential in the substrate. This may be because the average redox potential is higher in River Hoenselva (601 mV) than in Skorgeelva (557 mV). Since it is higher in Hoenselva, it may be that the conditions were too favorable to have an impact on the benthic macroinvertebrate community. However, the redox potential did have an impact on how the benthic macroinvertebrate community distributes itself.

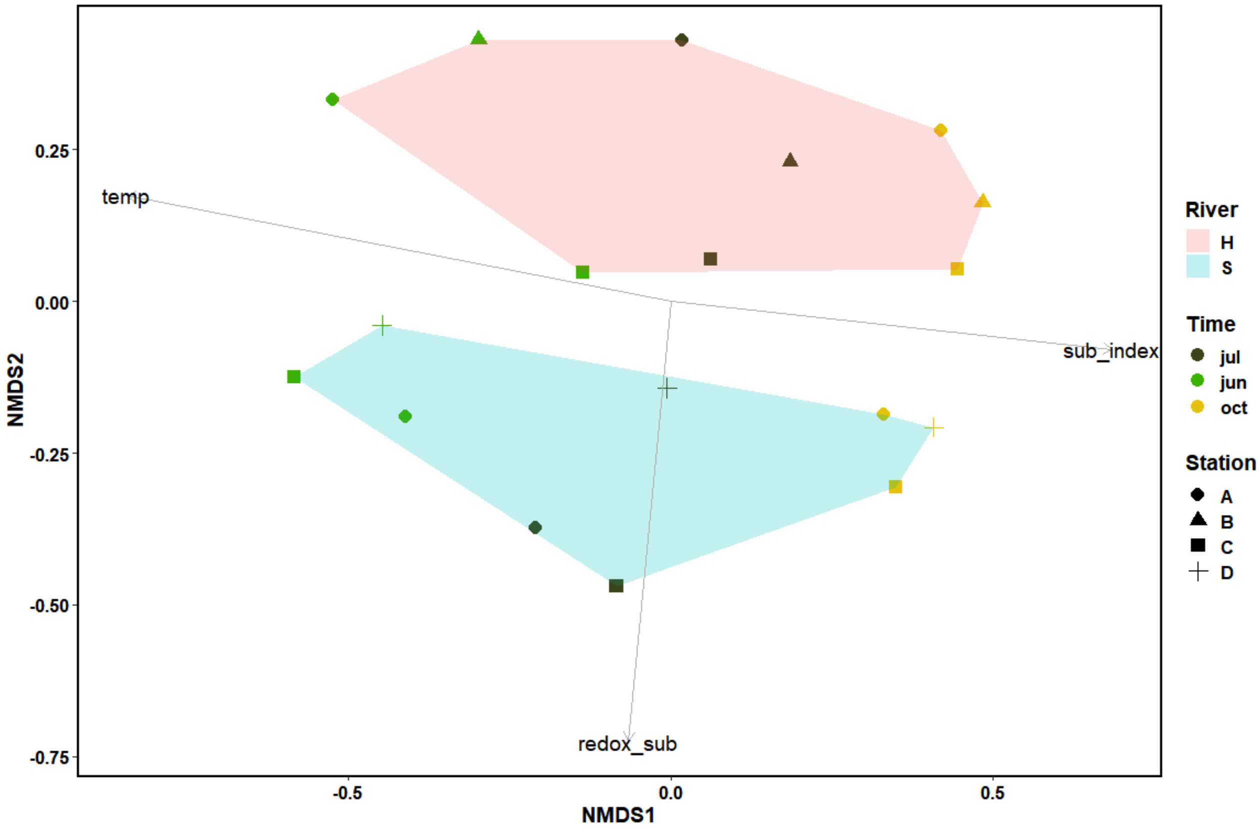

Figure 16 Non-metric multidimensional scaling (NMDS) analysis of benthic species distribution based on hydromorphological parameters across all stations in River Hoenselva and River Skorgeelva during June, July, and October.

3.4.2 Correlation

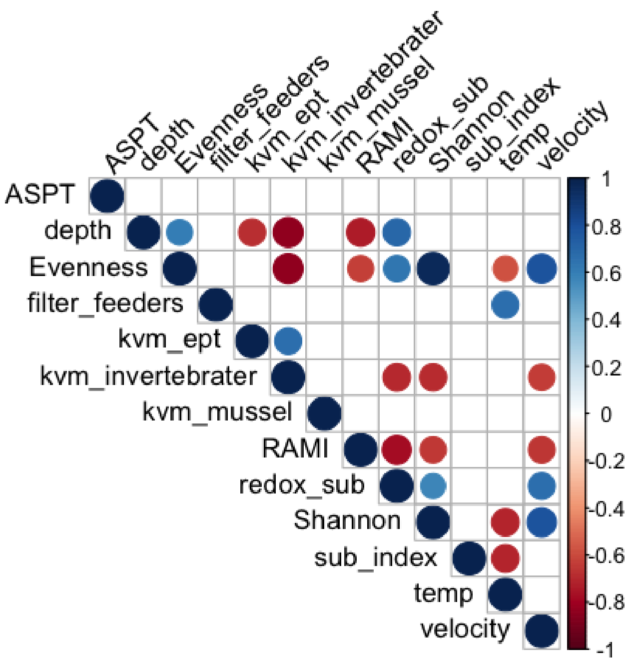

Figure 17 Correlation plot over our variables, from River Hoenselva and River Skorgeelva. The dots show the correlations which have a p-value <0.05, where blue indicates positive correlation and red indicates negative correlation. (1) **ASPT** = Average score per taxon (2) **depth** (cm) (3) **Evenness** index

(4) **filter_feeders** = % of filter feeding macroinvertebrates (5) **kvm_ept** = density of EPT (individuals per m^{-2}) (6) **kvm_invertebrates** = density of benthic invertebrate fauna (individuals per m^{-2}) (7) **kvm_mussel** = density of freshwater pearl mussel (*Margaritifera margaritifera*) (individuals per m^{-2}) (8) **RAMI** = River acidification macroinvertebrate index (9) **redox_sub** = redox potential E_H (mV) in the substrate (10) **Shannon** index (11) **sub_index** = index of the substrate size (12) **temp** = temperature (°C) (13) **velocity** (m s^{-1}).

Figure 17 shows a correlation plot of the variables from both rivers. The plot reveals that temperature, velocity, depth, and redox potential in the substrate are the key significant variables correlated with the various benthic invertebrate variables.

Starting with the variables that were significant in the NMDS modeling, we observe that temperature and redox potential in the substrate show significant correlations with many variables. Temperature was significantly negatively correlated with the Shannon index and Evenness index, and weakly positively correlated with filter feeders. The redox potential in the substrate was significantly negatively correlated with the density of benthic organisms, as well as the RAMI index. The redox potential was also significantly positively correlated with Evenness and the Shannon index. The substrate index was not correlated to any of the biological variables in this plot.

Among the other variables, we observe that velocity was significantly positively correlated with both the Shannon index and Evenness, but negatively to the density of macroinvertebrates. Another interesting variable is depth, which was significantly negatively correlated with the density of benthic organisms, RAMI index as well as the density of EPT taxa. Depth was also significantly positively correlated to Evenness.

We also see that the density of freshwater pearl mussel was not significantly correlated with any of the other variables, despite this being expected. It may be because this plot is based on both rivers, the combination of the data might have lost any connection shown when separating into rivers. The ASPT index was not correlated with anything.

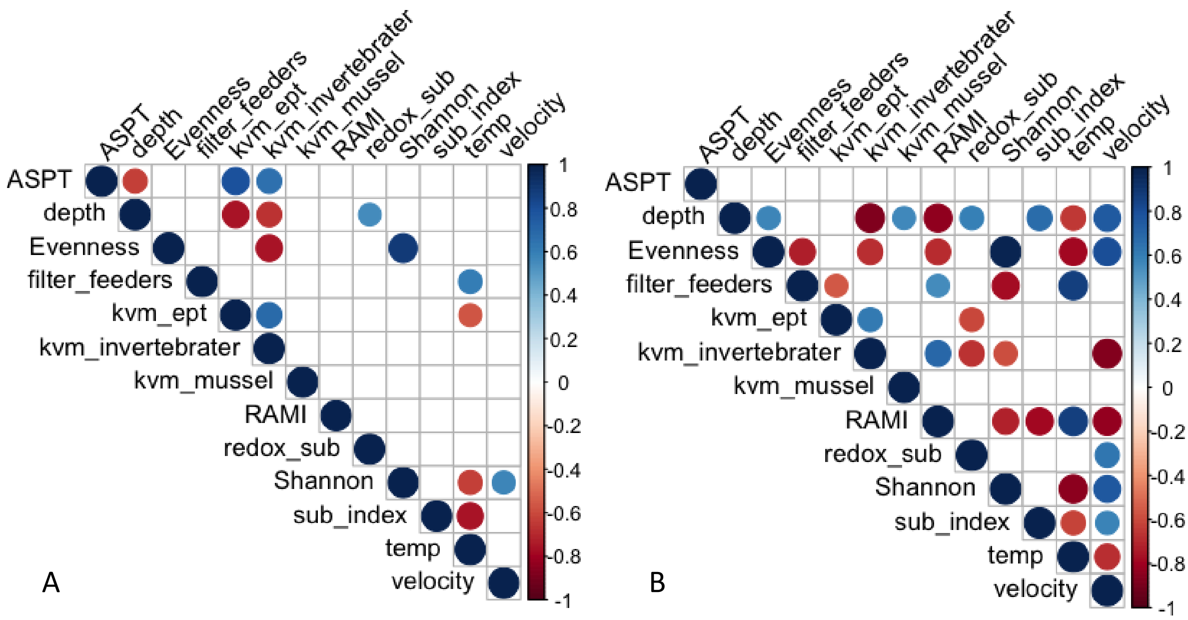

Figure 18 Correlation plots over our variables separated into rivers, A = River Hoenselva and B = River Skorgeelva. The dots show the correlations that have a p-value <0.05, where blue indicates positive correlation and red indicates negative correlation. (1) **ASPT** = Average score per taxon (2) **depth** (cm) (3) **Evenness** index (4) **filter_feeders** = % of filter feeding macroinvertebrates (5) **kvm_ept** = density of EPT (individuals per m^{-2}) (6) **kvm_invertebrates** = density of benthic invertebrate fauna (individuals per m^{-2}) (7) **kvm_mussel** = density of freshwater pearl mussel (*Margaritifera margaritifera*) (individuals per m^{-2}) (8) **RAMI** = River acidification macroinvertebrate index (9) **redox_sub** = redox potential E_H (mV) in the substrate (10) **Shannon** index (11) **sub_index** = index of the substrate size (12) **temp** = temperature (°C) (13) **velocity** (m s^{-1}).

Figure 18 depicts two correlation plots for each river separately. Starting with River Hoenselva, the temperature was significantly weakly negatively correlated with the Shannon index and the density of EPT individuals. Temperature was also significantly positively correlated with filter feeders. For the redox potential and the substrate index, we only see significant correlations with other hydromorphological variables. Among other variables, we see that the depth was significantly negatively correlated with the density of macroinvertebrates and EPT individuals, and the ASPT index. We observe no correlations between the density of freshwater pearl mussels and any of the other variables in Hoenselva.

In River Skorgeelva, we observe that temperature was significantly negatively correlated with the Evenness and Shannon indexes, and positively correlated with filter feeders and the RAMI index. For the redox potential in the substrate, we see a significant negative correlation with the density of macroinvertebrates and EPT individuals. The substrate index was negatively correlated with the RAMI index. As for the other variables, we see that the depth was significantly negatively correlated with the density of macroinvertebrates and the RAMI index, and positively correlated with the Evenness index and the density of freshwater pearl mussels. Velocity was also significantly negatively correlated with the density of macroinvertebrates and RAMI index, and positively correlated to the Evenness index and the Shannon index.

We see that there are some differences between the two rivers. In River Hoenselva, there are a significant negative correlation between the temperature and the density of EPT that we do not have in River Skorgeelva. However, both rivers showed a significant correlation between temperature and filter feeders and the Shannon index. In Hoenselva, there was no significant correlation between the redox potential in the substrate and the substrate index and other biological variables. However, in Skorgeelva we observed a significant correlation between the redox potential and the density of macroinvertebrates and EPT individuals. The substrate index in Skorgeelva was also significantly correlated to the RAMI index. We saw little to no correlation between the freshwater pearl mussel density and the other variables in both rivers, with only a significant correlation with depth in Skorgeelva.

4 Discussion

4.1 Interpretation of benthic macroinvertebrate data

Generally, we found a relatively low diversity of benthic macroinvertebrates in both rivers, regarding the Shannon Index (**Table A 3**). This does not necessarily mean that the rivers are affected by environmental degradation (Ravera, 2001). The high proportion of Chironomidae (42% of total) and *Baetis rhodani* (16% of total) might explain the low values of both Shannon and Evenness, as we have some species that clearly dominates the samples (Krebs, 1989). The low diversity might also be explained by the natural selection of species in colder water bodies (Ravera, 2001).

Chironomidae larvae clearly dominated in the June samples and were very numerous (**Table A 7**). This family is used as an indicator for eutrophication, as it is often abundant in areas affected by sewage discharge (Machado et al., 2015). However, based on the calculated eutrophication index (ASPT), both River Hoenselva and River Skorgeelva was classified with 'good' ecological condition (**Table A 3**) according to the Norwegian Water Framework Directive (2018). Therefore, it is conceivable that the high numbers of Chironomidae larvae is due to other factors, such as seasonality and temperature (Ciemiński and Zdanowski, 2009), rather than eutrophication.

A substantial proportion of EPT taxa were generally recorded (around 70% of total taxa) (**Table 9**), with Trichoptera being the dominant group. This is not very surprising, as this group usually dominates among the EPT taxa (Morse et al., 2019). EPT densities are often used as indicators of good water quality in rivers (Mason, 1996). The high proportion of EPT taxa in the samples suggests that the rivers was minimally affected by physical and chemical changes, eutrophication and/or acidification (Raddum and Fjellheim, 1984; Thorne and Williams, 1997). EPT taxa may also indicate that the rivers was oxygen-rich and of good quality (Ambelu et al., 2010; Mason, 1996; Selvanayagam and Abril, 2015). The number of EPT taxa was similar between the two rivers (54 in River Hoenselva, 55 in River Skorgeelva), indicating no apparent difference in water quality.

Baetis rhodani (Ephemeroptera order) was particularly abundant in both rivers. This species thrives in habitats dominated by rocks and gravel (Vilenica et al., 2018), which was the dominant substrate type in both rivers. It is also sensitive to morphological changes in the habitat, i.e. homogenization of the substrate (Šumanović et al., 2024). Our results showed no correlation between the substrate index and density of the EPT, nor the density of benthic macroinvertebrates (**Figure 17; 18**). We assume this is because our substrate index was quite uniform (6.44 – 6.76) throughout both rivers, and across stations. The substrate index is a number for how coarse the substrate is, meaning that a higher index shows a coarser substrate. If we had a bigger difference in substrate e.g. more shingle bars, we might have seen correlations with the substrate index.

Baetis rhodani can be used as an indicator for acidification, as it is highly sensitive (Andrén and Eriksson Wiklund, 2013; Raddum and Fjellheim, 1984). In both rivers, the high number of *Baetis rhodani* and the acidification index (RAMI) demonstrated the good water quality of the sampling sites. We also see that the pH from previous studies show that there is little to no issues with acidification (Larsen and Berger, 2009; Larsen and Magerøy, 2020). This means that both rivers was good habitats for many acidification-sensitive species.

Regarding the functional feeding groups, we found a varying proportion of filtering macroinvertebrates in our samples (0.1 – 24.6%) (**Table A 5**). Filter feeders collect their nutrients from the water column using nets or mouth-part structures (Ramírez and Gutiérrez-Fonseca, 2014). The filter feeders in River Hoenselva and River Skorgeelva was significantly positively correlated with water temperature. This might be because many filter feeders have been shown to have high tolerance for warm temperatures (Tomczyk et al., 2022).

River Hoenselva had a lower density of benthic macroinvertebrates (including EPT taxa), somewhat higher diversity, and a higher proportion of filter feeders compared to River Skorgeelva. To assess the composition of the benthic community in our rivers, it may be useful to look at older surveys from the river itself, as well as from nearby rivers.

Johansen (1990) collected benthic macroinvertebrate samples in the water column at two stations from May to October 1989 in River Hoenselva. He found that the dominant groups of benthic macroinvertebrates were black flies (Simuliidae), Chironomidae, and other Diptera species. This is consistent with our results, which may suggest that conditions in the river have not significantly changed since 1989. However, we also found a large number of other species, especially within the EPT order. Johansen (1990) do not mention any findings of EPT taxa, which may be due to the sampling method, as he used a drift net to collect benthic macroinvertebrates in the water column, while we took samples by digging in the substrate and sampling with Surber.

The water chemistry in River Hoenselva has been shown to be relatively stable. From 1996 to 2008, there have been few changes, and most of the changes have occurred in the lower reaches, such as the increase in nitrate and phosphorus concentrations (Larsen, 2017). Since there hasn't been much change, it can be assumed that the benthic communities from the 1990s and from this study stay relatively similar.

A nearby river to River Hoenselva is River Bingselva, which also flows into River Drammenselva. Brittain et al., (1985) mapped the benthic community in the river and found many acidification-sensitive families, and Plecoptera species. Comparing with Hoenselva today, our RAMI values show that Hoenselva is not currently affected by acidification. Together, this can indicate that the rivers in the area have not been affected by this for some time. This is also reflected by the water quality in Hoenselva from previous studies (Larsen, 2002; Larsen and Berger, 2009). We also found 54 EPT taxa, which indicates good water quality (Ambelu et al., 2010; Selvanayagam and Abril, 2015).

During previous studies conducted by Norconsult AS (2023) in River Skorgeelva during late summer and fall of 2022, a large number of EPT taxa were found, with Ephemeroptera, Plecoptera, and Trichoptera all well represented in the sample. The most numerous of the three was Ephemeroptera. Additionally, they calculated an ASPT of 6.4, similar to our results (5.25 – 7.05). Norconsult took their samples a bit downstream from our lowest station, where algal growth in the water and certain indicator species of algae were also observed, which reduced the ecological condition to 'good' (Norconsult AS, 2023). This suggests that ecological condition was unchanged from summer of 2022 to 2023.

Water quality samples from River Skorgeelva were conducted by Larsen and Magerøy (2020) in August and October of 2019, which classified the river to 'very good' ecological condition (Direktoratsgruppen vanndirektivet, 2018). This is also reflected in the results that we found within our ASPT and RAMI indexes. Both indexes were high (ASPT >5.0, RAMI > 5.0), which indicates a 'good' and 'very good' ecological condition respectively (Direktoratsgruppen vanndirektivet, 2018). We also found 55 different EPT taxa, which also indicated good water quality (Ambelu et al., 2010; Selvanayagam and Abril, 2015).

Norconsult AS (2023) examined a total of 51 stations in rivers and streams in southeastern Norway, regarding the benthic macroinvertebrate community and periphytic algae. Only 3 of the stations were found to be in 'very poor' condition, and they were all located in streams in agricultural areas. 4 stations were in 'poor' condition, 17 in 'moderate', 17 in 'good', and 10 in 'very good' ecological condition. During this survey, potential mussel populations were not evaluated. In comparison, our studies showed that River Hoenselva and River Skorgeelva was in similar or better ecological condition than many of the nearby watercourses. However, we did not sample periphytic algae, and this could have influenced the ecological condition.

In Norway, one of the most significant threats to biodiversity is eutrophication. Although, rivers that was originally nutrient poor might benefit from the increasing eutrophication, many species react negatively to this. Increased amounts of phosphorus and nitrogen can lead to increased production of plants and algae in the water, which contributes to reducing the amount of oxygen and light penetration and increasing sedimentation (Schartau et al., 2010). Even though this is a common problem in Norway, the macroinvertebrate community in our rivers appeared to be minimally affected. Relatively high ASPT values indicated 'good' ecological condition regarding eutrophication (Direktoratsgruppen vanndirektivet, 2018). Thus, there is a high number of species that are sensitive to eutrophication in both of our rivers (Armitage et al., 1983).

Areas in Southern Norway have been affected by acidification due to man-made emissions of nitrogen and sulfur in Europe (Johannessen, 1995), and this has been the second most important reason for biodiversity loss in Norway. Although improvements have been noted in this area because of several international agreements, a significant proportion of water bodies were still clearly affected by acidification in Norway in 2010 (Schartau et al., 2010). Both of our rivers had benthic macroinvertebrate communities consisting of many acidification-sensitive species, and thus received very high values for RAMI (Direktoratsgruppen vanndirektivet, 2018 Appendix).

Freshwater pearl mussels are used in Norway as a threshold indicator species, used to assess the ecological status of rivers. Documentation of juvenile mussels determines the condition class to which the river belongs (Direktoratsgruppen vanndirektivet, 2018). Earlier studies in River Hoenselva showed that the freshwater pearl mussel population was unstable, and that the recruitment was unsatisfactory. All three studies also noted that there was no recruitment in the lower reaches of the river, and lacking in the upper reaches (Larsen, 2002; Larsen and Berger, 2009; Larsen and Magerøy, 2019). In River Skorgeelva the population estimates suggest that Skorgeelva has a large population (Gregersen, 2018; Larsen and Magerøy, 2020;

Sandaas and Enerud, 2009). However, Larsen and Magerøy (2020) found that the recruitment was poor because there were no mussels smaller than 20 mm. Both of our rivers had findings of mussels both smaller than 50 mm and 20 mm (**Table 6; 8**). Based on the freshwater pearl mussel data and other biological quality elements (ASPT and RAMI), both Hoenselva and Skorgeelva are classified under 'good' ecological condition (Direktoratsgruppen vanndirektivet, 2018).

The freshwater pearl mussel recruitment levels in our rivers were not sufficient to maintain the mussel populations (Larsen, 2017). In River Hoenselva, 4 mussels under 20 mm were found. Our samples were taken in the upper reaches, where earlier studies have shown a large mussel population, compared to the lower reaches (Larsen, 2002; Larsen and Berger, 2009; Larsen and Magerøy, 2019). Thus, our studies do not include the areas where the recruitment is known to be poor, and we cannot assess ecological status to the entire river. In River Skorgeelva, one mussel under 20 mm was found in the lower reaches. No mussel under 20 mm was found in the upper reaches, which were unexpected due to the higher number of mussels visible on the surface (**Table 8**). However, the one mussel indicated a recent recruiting population.

4.2 Influence of hydromorphological variables on benthic community

The results of the NMDS model show that temperature, redox potential in the substrate and the substrate index significantly affect how the benthic macroinvertebrate community is organized (**Figure 16**). We also found significant correlations between the benthic community and some hydromorphological variables. There were certain hydromorphological differences between the rivers that are worth noting. River Hoenselva had significantly lower water velocity, deeper water, and higher redox potential in the substrate, compared to River Skorgeelva. Additionally, Hoenselva had higher diversity (Shannon index).

The diversity of the benthic communities was significantly negatively correlated with the temperature in both rivers (**Figure 17; 18**). This is supported by the literature, which show that water temperature often has a negative impact on the species diversity, as well as the density of benthic macroinvertebrates (Heth and Bowles, 2022; Krepski et al., 2014; Mehler et al., 2015). Certain groups of macroinvertebrates are tolerant to increased water temperatures, such as Chironomidae, which have been shown to dominate the benthic community under moderately high water temperatures (<28°C) (Ciemiński and Zdanowski, 2009). We found a lot of this taxa group in our samples, especially in the June-samples when the water temperature was also at its highest (**Figure 4; 7**). Simultaneously, this could also be related to natural seasonal variations in the benthic community. A study from Ireland recorded a clear seasonal variation in the benthic community. They found that the density of benthic organisms increases throughout the late summer and autumn, and they also found a higher species diversity and abundance of particularly shredders and filter feeders (Giller and Twomey, 1993). One reason for the higher diversity in the fall may be that the conditions in the river are more stable, and it coincides with the natural life cycle of the species (Huttunen et al., 2022). This aligned with our results, that showed an increasing Shannon index later in summer (**Table A 3; Figure A 1**). There was no significant difference in water temperature between River Hoenselva and River Skorgeelva (**Table 3**), and the benthic macroinvertebrate communities appear to be similarly affected by this variable (**Figure 16**).

All our redox measurements from the interstitial zone were above 400 mV (**Figure 15**), indicating favorable conditions for the benthic fauna and for the recruitment of freshwater pearl mussels (Chakrabarty and Das, 2006; Geist and Auerswald, 2007; Knott et al., 2019). In our rivers, redox potential in the substrate and water velocity followed a similar pattern, with the lowest values in June and the highest values in July (**Figure 13; 15**). Our results also showed a significant positive correlation between water velocity and redox potential in River Skorgeelva (**Figure 17**). Fine particles settling on the riverbed during periods of low water flow contributes to reducing the amount of oxygen penetrating the substrate (Geist and Auerswald, 2007; Richards and Bacon, 1994). Periods of higher water flow, such as the extreme weather event "Hans" in early August (Granerød et al., 2023) can wash away this fine material, making the substrate less compact (Geist and Auerswald, 2007). Thus, periods of increased water flow can contribute to improving habitat quality for freshwater pearl mussels as well as other invertebrates (Geist and Auerswald, 2007; Jones et al., 2012).

The measurements of the redox potential from River Hoenselva and River Skorgeelva showed higher values in this study compared to the previous investigation in 2018 and 2019 respectively (Larsen and Magerøy, 2020, 2019). The reason for this might be that we took measurements over several months, in addition to the rivers being exposed to a period of higher-than-normal flow during summer (https://sildre.nve.no/map).

Our results showed that there was a weak significant positive correlation between the redox potential in the substrate and the diversity of the benthic community, for both rivers combined (**Figure 17**). This is supported by the literature, which states that the oxygen content in the interstitial zones is important for the diversity of the benthic community (Ding et al., 2016; Palmer et al., 1997; Richter et al., 2016). However, there was no observed correlation between Shannon index and redox potential when looking at the rivers separately (**Figure 18**). The redox potential in the substrate seemed to affect the benthic community in River Skorgeelva to a greater extent than it did in River Hoenselva. Therefore, we cannot disregard that this was due to lower measurements of redox potential in Skorgeelva compared to Hoenselva.

Knott et al., (2019) conducted a study to examine the effect of erosion control in the catchment area and its impact on the benthic community in an area in Germany. They found that the structure and composition of the entire aquatic community in the river could be explained by differences in redox potential between the free water masses and the interstitial zones in the substrate. However, we found a significant negative correlation between redox potential in the substrate and the density of macroinvertebrates in both rivers combined and in River Skorgeelva (**Figure 17; 18**). This may be related to the high number of Chironomidae larvae which increased the densities of macroinvertebrates in early summer. Several of these species are tolerant to low levels of dissolved oxygen (Mantilla et al., 2018), and they were numerous in June when the lowest values of redox potential were measured.

Heterozygosity and complexity in the substrate have also proven to be crucial for diversity in rivers. Different taxa show different preferences for substrate type and position in the river (Buss et al., 2004). Jowett et al., (1991) explored microhabitat preferences for various macroinvertebrate taxa in New Zealand and found that none of the groups studied preferred fine substrates such as sand and fine gravel. Instead, most species were found in coarse sand and stone substrates. This aligns with our results, which showed that the benthic community was significantly affected by the substrate index (**Figure 16**). Our substrate index is a number for how coarse the substrate is, meaning that a higher index shows a coarser substrate. Our indexes were mostly around 6-7, meaning that the substrate had a lot of coarse gravel and cobbles (**Table 2; 4**). However, only RAMI showed a significantly negatively correlation with the substrate index in River Skorgeelva (**Figure 17**). We expected to see a relationship between substrate and density of benthic macroinvertebrates, but this was not evident in the correlations. This could be because the indexes we obtained appear quite similar, and thus may not show significant variations in benthic community composition.

A study by Jones (2013) investigated benthic macroinvertebrate community composition in both regulated and natural rivers across Canada. They concluded that abiotic factors like water velocity and depth are the primary determinants of the community's composition and structure.

Water velocity and Shannon index was significantly positively correlated in both rivers combined, as well as in each river separately (**Figure 17; 18**). This may be because the June samples had the lowest water velocity and were dominated by Chironomidae larvae. As water velocity increases in July and the number of Chironomidae decreases, we consequently get a higher Shannon index. It may also be that diversity generally increases in late summer and autumn compared to early summer due to natural variations (Giller and Twomey, 1993), which also aligns with when water velocity was low in our rivers (**Figure 13**). Water velocity was also significantly negatively correlated with the density of macroinvertebrates in both rivers combined, as well as in River Skorgeelva separately (**Figure 17; 18**). This may also be related to the fact that the density of Chironomidae larvae decreases after the June samples. Jones (2013) suggests that this could be a natural effect, as they found that several of the larger family groups (EPT and Diptera) often occurred in the shallower and slower-flowing areas.

We found a strong significant negative correlation between density of macroinvertebrates as well as density of EPT taxa and depth in both rivers combined (**Figure 17; 18**). Density of EPT taxa was not correlated with depth in River Hoenselva individually. The correlation in both rivers showed that the diversity of benthic macroinvertebrates in our rivers increased with decreasing depths. This might be due to a greater accumulation of resources (organic material) where it was shallower, and the water velocity was lower (Jones, 2013). Benthic macroinvertebrates may also inhabit shallower areas of the river to avoid shear stress and unstable substrate during periods of high water velocity (Rempel et al., 2000, 1999). We also assume it might be due to the decrease in Chironomidae larvae in July when the depth was higher (**Figure 14; Table A 7**). This is also reflected in that the Evenness index was positively correlated with the depth (**Figure 17**). The Evenness index demonstrates that the species was more evenly distributed when also considering their abundance (Wilsey and Potvin, 2000), so when the Chironomidae larvae decreases in July, the Evenness in turn gets higher.

There was a significant difference in depth in our rivers, with River Hoenselva being the deepest river (**Figure 14**). However, the river with the most correlations related to depth was River Skorgeelva (**Figure 18**). An interesting correlation in Skorgeelva was the positive correlation between the depth and the density of freshwater pearl mussels. Skorgeelva was generally shallower than Hoenselva, and this may explain why mussels only correlate with depth here. Previous studies in Skorgeelva have shown that mussel populations have been threatened by drought, risking a substantial number of mussels drying out (Bløndal, 2018; Sandaas and Enerud, 2015).

Based on the literature, we expected the presence of freshwater pearl mussels to lead to higher densities and diversity of benthic organisms due to the keystone species effect of unionid mussels. Unionids significantly rework the substrate, contributing to more water in the substrate, homogenizing the structure of the substrate, and increasing the amount of oxidized sediment (McCall et al., 1979). At the same time, they contribute to the filtration and purification of the water and biodeposit nutrients into the substrate. All these functions lead to optimal living conditions for benthic organisms, with good habitats and availability of nutrients (Geist, 2010; Nath et al., 2023). A study from South America conducted by Simeone et al., (2021) found a higher number of individuals and species of benthic organisms at sites with freshwater mussels. Particularly, there was a high number of Trichoptera in these areas, and the dominant functional feeding groups were shredders, predators, and collectors from this order. Additionally, a study from the US showed that the density of unionid mussels correlates positively with the density of macroinvertebrates. Groups such as Oligochaeta, Chironomidae, Ephemeroptera, and Trichoptera were especially dominant in areas with a higher density of mussels (Vaughn and Spooner, 2006).

We were unable to find a correlation between the density of freshwater pearl mussels and the density or diversity of the benthic community (**Figure 17; 18**). A reason could be that the conditions in the river were sufficiently favorable for benthic macroinvertebrates that the presence of mussels did not influence the benthic community as we anticipated. If the conditions had been different, we might have observed a greater effect. However, there is a study from Germany, conducted by Richter et al., (2016), revealing that freshwater mussels did not affect the diversity, taxonomic composition or diversity of the benthic community. This study, however, reports methodological insufficiencies and intense agriculture in nearby areas, which should be taken into consideration. The same study concluded that abiotic factors such as temperature, dissolved oxygen, and redox potential in the substrate could potentially better explain the diversity in the benthic community (Richter et al., 2016).

4.3 Weaknesses of the study

The main weakness of our study is the lack of data. Insufficient data prevented us from completing the models as intended. Our models required the river to be a random factor, with stations nested within the river. Additionally, we chose to use the dataset where measurements were taken for each station per month (e.g., River Hoenselva station A in June, A in July, and A in October etc.). This decision was made because the Surber samples alone provide a limited view of the benthic community and combining them per station might provide a more accurate picture, even though we might lose some of the variations showed on a smaller scale. Consequently, we had only 18 observations, which is not a large dataset. With such a small amount of data, the degrees of freedom for the models quickly became depleted. Despite attempting various model selection techniques to streamline the models, and data down to cell level, we were unable to find a solution that worked. We had originally intended to sample more stations in more rivers but had to limit our study due to resource limitations. In addition, we lost one sampling round due to the extreme weather “Hans” (Granerød et al., 2023). If we had managed to follow the original plan, we would have had more data and a better chance of running our models.

Additionally, gathering data over several years may reveal larger variations in rivers, which could be valuable for understanding long-term trends. It's worth noting that the high waterflow in 2023 (https://sildre.nve.no/map) might have limited our ability to detect effects of variables that are sensitive to poor conditions, i.e. prolonged drought periods. Moreover, expanding the study to include other rivers, especially those with greater differences in mussel populations could provide valuable insights into ecosystem dynamics.

Our original design was focused on the influence of hydromorphology on freshwater pearl mussel recruitment and benthic macroinvertebrates. However, since we did not obtain much data on mussel recruitment from the surveys, we had to adjust our research questions. If we had known that we would not focus on mussel recruitment, we could have planned for more Surber samples. We could have also opted for a stratified sampling approach instead of a randomized one. In this case, we could have selected routes with larger differences in mussel densities, to increase variation between samples. With greater variation, the data might reveal relationships that are not evident with our current dataset. However, it's important to note that making such a choice would introduce a bias, and this could also affect how our results would translate to natural ecosystems with less variability.

When using correlations to interpret data, one must be careful of spurious correlation. Spurious correlation can lead to misleading interpretations and can create relationships that are false (Berryman and Turchin, 1997; Håkanson and Stenström-Khalili, 2009). To counteract this, further testing is needed. However, since our GLMMs did not work we were unable to check our finds.

Another weakness of the study could be the use of macroinvertebrates as indicators, which is a topic of debate in ecological research. One reason for this is the inherent variability of macroinvertebrates across different seasons and spatial scales. Due to these variations, it can be challenging to detect the need for conservation action until it is almost too late. When such discoveries are made too late, conservation strategies often prove ineffective because they require time to be implemented (Koop et al., 2011). The spatial variation can also be difficult when executing studies on invertebrates. Studies conducted on different spatial levels such as local or landscape scales might draw different conclusions (Sandin and Johnson, 2004). Our study only used variables on a local scale, meaning that it might be biased in this direction. However, our intentions with the study were more targeted towards the local scales.

For macroinvertebrates to be used as indicators, it is essential to identify species at the species level. This is because species may react differently to pollution than their overall genera. It is also necessary to consider life-history traits since fluctuations in population size can vary naturally, necessitating a focus on the species level. However, species-level identification requires more time and expertise, often resulting in higher costs (Rosenberg et al., 1986). New methods, such as eDNA, can help simplify and streamline the identification of benthic fauna (Fernández et al., 2019).

Although the use of benthic macroinvertebrates as indicators is debated, it can still be very useful for identifying stressors on river habitats (Markert et al., 2022; Urbanič et al., 2020), as they are a large, diverse, and important group of organisms (Chaloner et al., 2009). Various indexes and indicators are used nationally and internationally to classify ecological condition (Dale and Beyeler, 2001; Direktoratsgruppen vanndirektivet, 2018; Mobasher et al., 2023; Rosenberg et al., 1986; Vergolyas et al., 2020).

Although it was not possible to confirm the interactions between our variables, we have nevertheless gained an overview of the composition of the benthic macroinvertebrate community and the ecological condition of the rivers, as well as some insight into the recruitment of freshwater pearl mussels. Our study also showed how various hydromorphological variables can play a role in the distribution of species in the benthic community, and we have gained insight into these conditions. The composition of benthic organisms was influenced by many different variables and complex interactions, but this study highlights the importance of available oxygen in the substrate, good temperature conditions, and substrate composition. At the same time, these variables were affected by other conditions in the river such as water velocity and depth.

5 Conclusion

The dynamic properties of running water also imply frequent changes in lotic benthic habitats, both in time and space. Thus, the variations in habitats within a stream might be larger than between streams. In addition, most of the benthic fauna in lotic environments are temporary water dwellers, which means that time of sampling also is of great importance. Other decisive factors are water chemistry, access to resources, predation, and other causes of death as absence of water for longer time periods during draught. For the freshwater pearl mussel, also presence of host fish for the glochidia, i.e. salmon or brown trout, are crucial. The glochidia are also relatively sensitive regarding the physical/chemical conditions in the substrate sediments, which they inhabit during the first 4-8 years of their lives. The physical, chemical, and biologic conditions, both in water and sediments, are also highly important for the quality and quantity of the remaining benthic fauna.

In our study we have only studied some of these crucial factors. Despite so, the study has been useful for mapping the composition of the benthic community in the rivers and the populations of freshwater pearl mussels, as well as assessing the ecological condition and potential stress factors in the river habitats. In addition, we have got an overview of some hydromorphological variables of importance for the composition of benthic communities.

Hopefully, our work is an important piece into the complicated large puzzle dealing with the numerous physical, chemical and biological factors of importance for the composition of benthic community in lotic environments.

6 References

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7 Appendix

Table A 1 UTM coordinates for the stations in River Hoenselva.

Station	UTM	North	East
A	32V	6628030	0546225
B	32V	6628230	0546429
C	32V	6628441	0546719

Table A 2 UTM coordinates for the stations in River Skorgeelva.

Station	UTM	North	East
A	32V	6567969	0562607
C	32V	6567569	0562711
D	32V	6565856	0563148

Table A 3 Overview of the maximum and minimum values for every index (Evenness, ASPT, RAMI, Shannon) in both River Hoenselva and River Skorgeelva.

River	Station	Index	Max Value	Min Value
River Hoenselva	HA	Evenness	0.873	0.185
	HB	Evenness	0.798	0.607
	HC	Evenness	0.815	0.432
Total		**Evenness**	**0.873**	**0.185**
	HA	ASPT	6.588	5.200
	HB	ASPT	6.500	4.857
	HC	ASPT	6.900	5.833
Total		**ASPT**	**6.900**	**4.857**
	HA	RAMI	5.708	5.057
	HB	RAMI	6.199	5.119
	HC	RAMI	6.012	5.068
Total		**RAMI**	**6.199**	**5.057**
	HA	Shannon	2.474	0.501
	HB	Shannon	2.453	1.692
	HC	Shannon	2.584	1.316
Total		**Shannon**	**2.584**	**0.501**
River Skorgeelva	SA	Evenness	0.737	0.270
	SC	Evenness	0.741	0.408
	SD	Evenness	0.703	0.284

Total		**Evenness**	**0.741**	**0.270**
	SA	ASPT	7.056	5.250
	SC	ASPT	6.455	5.444
	SD	ASPT	6.895	5.666
Total		**ASPT**	**7.056**	**5.250**
	SA	RAMI	6.141	5.371
	SC	RAMI	6.240	5.494
	SD	RAMI	6.458	5.149
Total		**RAMI**	**6.458**	**5.149**
	SA	Shannon	2.450	0.730
	SC	Shannon	2.256	1.131
	SD	Shannon	2.371	0.806
Total		**Shannon**	**2.450**	**0.730**

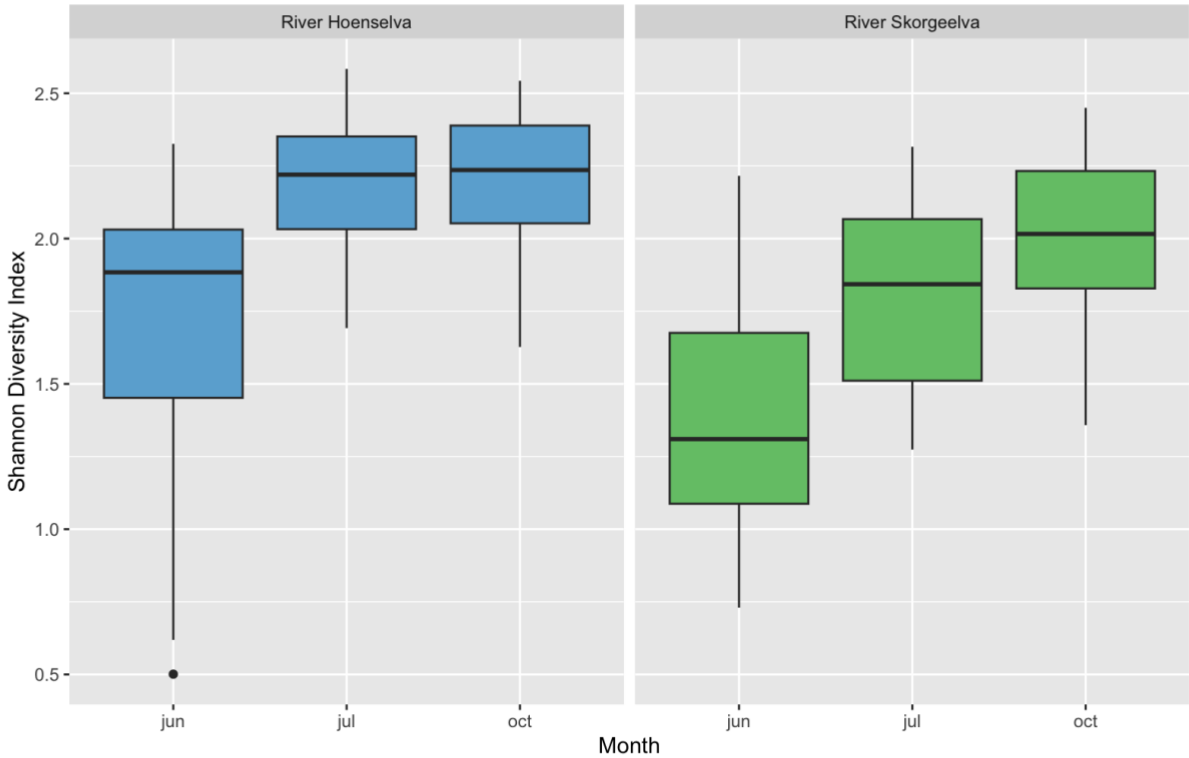

Figure A 1 Boxplot showing Shannon Diversity Index, throughout the summer of 2023, in River Hoenselva and River Skorgeelva.

Table A 4 The number of EPT (Ephemeroptera, Plecoptera and Trichoptera) species in River Hoenselva and River Skorgeelva.

Order	Number of taxa in River Hoenselva	Number of taxa in River Skorgeelva
Ephemeroptera	12	13
Plecoptera	15	18
Trichoptera	27	24

Table A 5 The reserve cells which were utilized in the field, with their original cell number and why they were selected.

	Reserve Cell	Original Cell	Reason
River Hoenselva	H06A1-6	H06A1-1	Unable to perform Surber sampling.
	H06B8-6	H06B8-1	Unable to perform Surber sampling.
	H09A4-3	H09A4-2	Unable to perform digging.
	H09A11-4	H09A11-3	Unable to perform digging.
	H09B8-2	H09B8-2	Unable to perform digging.
	H010A7-1	H010A7-2	Unable to perform Surber sampling.
	H010B7-1	H010B8-4	Unable to perform Surber sampling.
	H010C2-3	H010C1-2	Unable to perform Surber sampling.
River Skorgeelva	S06C9-2	S06C9-4	Unable to perform Surber sampling.
	S06D5-2	S06D5-1	Unable to perform Surber sampling.
	S09C6-3	S09D6-5	Unable to perform digging.
	S010C4-2	S101C5-5	Unable to perform Surber sampling.
	S010D2-2	S010D-6	Unable to perform Surber sampling.
	S010D6-1	S010D6-4	Unable to perform Surber sampling.

Table A 6 The percentage (%) of the different functional feeding groups from each station distributed in months in River Hoenselva and River Skorgeelva.

Stations	Shredders	Sediment - feeders	Grazers	Active Filter Feeders	Passive Filter Feeders	Wood Feeders	Predators	Cell Piercers	Parasites	Others	Not Classified
H06A-tot	2.236	31.597	23.085	15.436	0.406	0	8.814	7.751	7.68	0.056	2.939
H06B-tot	4.678	33.933	33.705	7.011	5.463	0.006	4.56	3.529	3.505	0.211	3.4
H06C-tot	4.416	32.262	29.995	8.221	8.109	0.056	5.744	4.186	4.111	0.038	2.863
H07A-tot	4.337	25.988	19.946	7.331	24.651	0.016	8.603	3.281	3.281	0.184	2.382
H07B-tot	3.311	29.719	30.712	4.603	15.563	0.124	6.714	2.301	2.301	0.182	4.47
H07C-tot	3.507	25.354	28.412	4.198	17.52	0	11.268	2.099	2.099	0.045	5.499
H010A-tot	2.522	34.827	27.913	8.877	4.15	0.018	9.888	4.403	4.38	0.082	2.939
H010B-tot	2.691	41.627	37.262	1.78	5.95	0.02	6.225	0.89	0.89	0.054	2.61
H010C-tot	2.027	35.691	36.525	1.97	11.619	0.014	5.024	0.985	0.985	0	5.161
S06A-tot	0.797	33.947	27.61	13.376	0.543	0.013	7.328	6.746	6.677	0.009	2.952
S06C-tot	1.394	35.541	29.854	12.724	0.137	0	6.698	6.339	6.339	0	0.974
S06D-tot	1.354	31.817	28.578	13.476	1.887	0.007	7.087	6.764	6.726	0	2.303
S07A-tot	1.788	30.968	22.809	12.871	1.369	0.014	12.337	6.395	6.395	0.095	4.959
S07C-tot	1.867	34.152	28.813	10.13	2.34	0.021	8.25	5.093	5.065	0.082	4.187
S07D-tot	3.212	30.21	30.093	9.044	6.536	0.047	7.096	4.537	4.522	0.078	4.627
S010A-tot	3.017	35.221	36.124	1.916	5.231	0.03	7.831	0.933	0.933	0.03	8.734
S010C-tot	2.261	42.895	40.038	0.31	1.25	0.011	4.772	0.155	0.155	0.076	8.079
S010D-tot	3.096	38.349	42.358	0.362	4.985	0.041	6.213	0.192	0.181	0.027	4.195

Table A 7 The taxon list with counted benthic macroinvertebrate individuals, from River Hoenselva and River Skorgeelva.

Species	River Skorgeelva									River Hoenselva								
	June			July			October			June			July			October		
	A	C	D	A	C	D	A	C	D	A	B	C	A	B	C	A	B	C
Ephemeroptera																		
Baetis sp.	56	58	0	1	0	0	0	3	7	0	1	1	5	5	11	0	57	104
Baetis muticus	115	129	24	25	15	16	117	129	119	8	2	21	4	11	13	121	189	203
Baetis niger	0	0	0	51	69	9	14	8	8	0	0	0	0	0	29	0	0	2
Baetis rhodani	128	128	264	27	45	148	732	1345	1695	94	321	340	109	280	264	93	404	691
Baetis digitatus	0	1	18	5	13	17	11	53	0	2	0	21	21	6	1	30	4	0
Centroptilum luteolum	6	1	0	1	1	2	0	0	0	15	4	3	0	0	2	0	0	0
Heptagenia sp.	1	4	10	0	0	1	0	0	0	0	2	11	1	1	0	0	0	0
Heptagenia sulphurea	3	1	0	1	0	8	17	31	84	3	3	3	5	4	13	126	44	39
Heptagenia joernensis	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Caenis luctuosa	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ephemerella ignita	706	537	372	62	109	58	0	1	0	159	31	33	28	6	20	0	0	0
Ephemera danica	0	0	0	1	0	0	0	0	0	0	0	0	16	0	0	0	0	0
Leptophlebiidae	1	0	0	0	0	2	1	1	6	2	1	2	12	7	1	29	0	4
Paraleptophlebia sp.	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Sum Ephemeroptera	**1016**	**861**	**688**	**174**	**252**	**264**	**892**	**1571**	**1919**	**283**	**367**	**435**	**201**	**320**	**354**	**399**	**698**	**1043**
Plecoptera																		
Siphonoperla burmeisteri	0	0	0	0	1	0	114	154	171	0	0	0	0	0	0	6	3	27
Brachyptera risi	0	0	0	0	0	0	0	1	6	0	0	0	0	0	0	0	0	0
Isoperla sp.	0	0	0	2	0	8	6	1	36	0	0	1	0	1	0	0	4	5
Isoperla grammatica	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0
Isoperla difformis	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	2	0	0
Amphinemura sp.	0	0	0	0	0	0	0	0	12	0	0	0	0	0	3	8	0	0
Amphinemura borealis	0	0	0	1	0	0	121	76	205	1	0	2	0	10	1	99	80	60
Amphinemura sulcicollis	0	0	0	0	0	0	29	2	14	0	0	0	0	1	2	20	6	1
Nemurella pictetii	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nemoura avicularis	0	0	0	0	5	17	0	1	0	0	0	0	3	0	0	0	0	0
Protonemura meyeri	0	0	0	0	0	1	3	0	3	0	0	0	2	3	2	6	5	9
Nemouridae	8	9	4	16	0	2	0	0	0	2	0	0	1	0	0	1	0	0
Leuctra sp.	9	0	10	0	0	0	6	3	9	0	28	123	0	0	0	0	1	2
Leuctra fusca	37	55	91	25	25	76	0	8	0	245	138	118	213	45	128	0	0	0
Leuctra hippopus	0	0	0	0	0	0	3	2	9	0	0	0	0	0	0	0	0	0
Capnia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	3
Capnopsis schilleri	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	8	11
Coleoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agabus sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Crambidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Orectochilus villosus	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0

Sum Plecoptera	**55**	**64**	**105**	**44**	**31**	**104**	**284**	**250**	**487**	**248**	**166**	**244**	**220**	**68**	**136**	**145**	**107**	**118**
Trichoptera																		
Rhyacophila nubila	2	1	1	2	1	8	2	0	11	6	6	16	11	12	26	9	7	28
Glossosomatidae	0	0	0	2	0	0	0	0	0	0	0	0	0	5	3	0	1	0
Agapetus ochripes	0	0	0	0	0	7	22	10	11	0	0	0	0	4	4	3	44	40
Oxyethira sp.	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0
Hydroptila sp.	8	0	4	0	1	1	0	0	1	7	1	4	0	0	0	1	0	0
Philopotamus montanus	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Polycentropus irroratus	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
Polycentropus flavomaculatus	22	14	6	116	41	26	39	16	13	12	6	13	53	10	92	46	4	19
Hydropsyche sp.	17	3	23	0	1	3	0	0	10	0	0	1	34	44	7	4	15	10
Hydropsyche pellucidula	2	0	0	32	5	48	35	23	52	2	0	2	21	18	123	8	7	20
Hydropsyche siltalai	1	2	2	0	0	2	44	8	117	10	2	6	13	21	20	20	9	21
Hydropsyche angustipennis	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	2	0
Lepidostoma hirtum	2	0	1	1	1	4	2	1	5	0	0	4	1	5	0	1	1	1
Limnephilidae	0	0	0	1	0	0	3	3	1	1	0	2	0	0	0	4	7	0
Limnephilus binotatus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anabolia nervosa	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetopteryx sp.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetopteryx villosa	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Tinodes pallidulus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tinodes waeneri	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Halesus radiatus	1	1	1	1	0	0	0	0	0	3	1	3	1	0	0	0	0	0
Wormaldia subnigra	8	0	10	0	0	1	0	0	0	0	5	3	0	0	3	0	0	0
Psychomyia pusilla	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Leptoceridae	25	2	24	16	7	28	10	2	20	1	0	7	3	4	13	0	2	3
Oecetis testacea	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Athripsodes sp.	0	0	0	8	0	0	0	1	0	0	0	0	0	0	1	0	0	0
Athripsodes commutatus	35	4	9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Ceraclea sp.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sericostoma personatum	1	0	0	2	0	2	3	0	4	0	5	3	0	4	4	2	1	2
Molannodes tinctus	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Chimarra marginata	0	0	0	0	0	11	0	0	0	0	0	0	5	3	0	18	1	2
Silo pallipes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Goera pilosa	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Sum Trichoptera	**127**	**28**	**81**	**183**	**57**	**142**	**160**	**64**	**246**	**50**	**31**	**65**	**143**	**133**	**296**	**116**	**102**	**150**
Odonata																		
Ophiogomphus cecilia	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Gomphus vulgatissimus	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
Cordulegaster boltonii	1	0	0	3	0	0	1	0	0	13	0	0	6	3	6	11	3	0
Sum Odonata	**1**	**0**	**0**	**3**	**0**	**0**	**2**	**0**	**0**	**14**	**0**	**0**	**7**	**3**	**6**	**11**	**3**	**0**
Diptera																		
Pericoma sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Tipulidae	4	5	5	0	0	9	1	1	2	0	0	2	0	1	0	0	0	0
Tabanidae	0	0	0	0	4	2	2	3	1	0	0	0	0	0	0	0	0	0

Simuliidae	5	2	58	1	27	127	62	16	91	8	86	164	411	141	225	32	70	216
Empididae	0	0	0	0	2	1	0	0	2	1	3	1	6	3	3	17	50	2
Chironomidae	3099	2732	2891	1341	738	1163	188	41	66	3005	598	876	606	278	374	745	133	208
Pediciidae	4	2	1	17	13	48	9	10	36	0	1	3	0	0	7	0	2	8
Ceratopogonidae	17	8	28	0	0	4	3	6	8	15	5	9	5	5	1	30	17	15
Antocha sp.	15	5	0	51	2	5	17	18	0	2	1	2	0	0	2	1	0	0
Sum Diptera	**3144**	**2754**	**2983**	**1410**	**786**	**1359**	**282**	**95**	**206**	**3031**	**694**	**1057**	**1028**	**428**	**612**	**825**	**273**	**449**
Coleoptera																		
Hydraenidae	11	25	95	3	8	57	14	16	77	3	31	55	2	8	21	5	9	30
Elmis aenea	54	16	30	59	16	33	144	152	75	99	44	40	30	24	76	16	8	32
Limnius volckmari	108	326	197	104	137	353	80	192	313	76	206	125	91	114	170	66	92	109
Curculionidae	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Elmidae	30	7	16	18	16	24	38	12	45	8	5	6	0	4	10	2	1	4
Sum Coleoptera	**203**	**374**	**339**	**184**	**178**	**467**	**276**	**372**	**510**	**186**	**286**	**226**	**123**	**150**	**277**	**89**	**110**	**176**
Mollusca																		
Unionoidea	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeriidae	1	2	0	1	0	0	1	0	0	3	0	0	3	0	0	2	0	0
Sum Mollusca	**1**	**2**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**3**	**0**	**0**	**3**	**0**	**0**	**2**	**0**	**0**
Arachnida																		
Acari	12	24	5	5	3	4	0	1	3	0	0	5	0	1	1	0	0	0
Sum Arachnida	**12**	**24**	**5**	**5**	**3**	**4**	**0**	**1**	**3**	**0**	**0**	**5**	**0**	**1**	**1**	**0**	**0**	**0**
Gastropoda																		
Gyraulus acronicus	0	0	0	0	3	2	0	1	2	11	18	4	12	6	4	7	4	0
Radix balthica	2	0	0	5	3	3	3	9	3	0	0	0	0	0	0	0	0	0
Ancylus fluviatilis	2	0	0	0	0	2	2	8	5	0	9	4	0	3	7	0	3	3
Sum Gastropoda	**4**	**0**	**0**	**5**	**6**	**7**	**5**	**18**	**10**	**11**	**27**	**8**	**12**	**9**	**11**	**7**	**7**	**3**
Annelida																		
Oligochaeta	88	217	93	80	122	213	103	235	264	86	126	94	99	76	89	105	184	119
Hirudinea	2	10	7	12	25	6	10	44	13	0	8	1	6	21	1	4	10	51
Sum Annelida	**90**	**227**	**100**	**92**	**147**	**219**	**113**	**279**	**277**	**86**	**134**	**95**	**105**	**97**	**90**	**109**	**194**	**170**
Megaloptera																		
Sialidae	0	0	1	2	0	10	0	1	1	1	0	1	5	0	0	1	0	3
Sum Megaloptera	**0**	**0**	**1**	**2**	**0**	**10**	**0**	**1**	**1**	**1**	**0**	**1**	**5**	**0**	**0**	**1**	**0**	**3**