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Ecological Implications of Road Construction in an Alum Shale Bedrock Area

A State Highway (Rv4) Case Study

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Preface and Acknowledgements

This report will serve as a 60 credits master thesis at the Department of Ecology and Natural Resource Management (INA) at the Norwegian University of Life Sciences (NMBU) in Ås, Norway. It will also be written under commission by the Norwegian Public Roads Administration (NPRA) with professor Thrond Haugen at NMBU-INA and associate professor Sondre Meland at NPRA as main and co-supervisor, respectively. The two of them took me on – with great pleasure and without hesitation I like to think – and gave me excellent guidance throughout this lengthy process. When I got stuck doing the statistics, they also came to my rescue. For their invaluable contributions to this paper I am very grateful.

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Now, let us get to the good stuff.

Abstract

The construction and use of roads and tunnels takes a toll on natural resources and especially the biological integrity of downstream freshwater environments. Consequently, aquatic biota is exposed to elevated levels of a wide range of contaminants, both natural and man-made, which in turn may have negative physiological and ecological effects on resident species. Since the autumn of 2013, the Norwegian Public Roads Administration (NPRA) has been constructing a new tunnel and associated roads on Rv4 Gran. The bedrock is partly alum shale, which is known to cause a host of environmental issues, such as acidic runoff and subsequent release of metals (e.g. aluminium) and radionuclides (e.g. uranium). These impacts are of great concern, but there are unfortunately considerable knowledge gaps as to their specific impacts at all spatial scales.

This study seeks to assess if the Rv4 project has imposed any negative ecological effects on benthic macroinvertebrate communities in streams and brooks inside impacted areas. In order to do so, benthic macroinvertebrates were sampled using kick-nets during spring and autumn in both 2013 (reference) and 2015 (impact). Macroinvertebrates are relevant bioindicators of environmental disturbance, and are frequently used to assess temporal changes in community composition and metal pollution history. Metals were analysed from mayflies (Ephemeroptera) and water quality variables were measured *in situ*.

In general, although to varying degrees, when compared with reference sites impacted areas were associated with: lower taxa richness, lower taxa diversity, much higher proportion of tolerant than sensitive species, lower ASPT index scores, elevated levels of mayfly metals, and a slightly decreased pH. ASPT index scores were found to be significantly negatively correlated with mayfly metal concentrations (all tested metals and a largely metal-loaded principal component first axis), supporting the use of ASPT index as a relevant indicator and response variable for metal contamination.

The results show that road and tunnel construction in areas with alum shale bedrock presents concerns related to ecological integrity and elevated metal concentrations, and measures ought to be taken in order to avoid compromising the health of recipient freshwater ecosystems.

Sammendrag

Utbygging og bruk av veier og tunneler har en negativ effekt på naturressurser og spesielt den biologisk integriteten til ferskvannsmiljøer som ligger nedstrøms. Akvatisk biota utsettes derfor for forhøyede nivåer av et bredt spekter av forurensninger, både naturlige og syntetiske, som igjen kan ha negative fysiologiske og økologiske effekter på lokale arter. Siden høsten 2013 har Statens vegvesen holdt på å bygge en ny tunnel og tilhørende veistrekning på Rv4 Gran. Berggrunnen der består delvis av alunskifer, som er kjent for å forårsake en rekke miljøproblemer, som for eksempel sur avrenning og påfølgende utslipp av metaller (f.eks aluminium) og radionuklider (f.eks uran). Disse påvirkningene er av stor bekymring, men det er dessverre betydelige kunnskapshull med hensyn til deres spesifikke effekter på alle størrelsesnivåer.

Dette studiet vil vurdere om Rv4-prosjektet har forårsaket negative økologiske påvirkninger på bentiske makroevertebrat-samfunn i elver og bekker innenfor påvirkede områder. For å gjøre dette så ble bentiske makroevertebrater samlet inn ved hjelp av sparkehåv i løpet av våren og høsten i både 2013 (referanse) og 2015 (påvirkning). Makroevertebrater er relevante bioindikatorer for miljøforstyrrelser, og blir ofte brukt til å vurdere tidsmessige endringer i samfunnssammensetning og historisk metallforurensning. Metaller ble analysert fra døgnfluer (Ephemeroptera) og vannkvalitets-variabler ble målt *in situ*.

Generelt, om enn i varierende grad, når sammenlignet med referanseområder så var påvirkede områder assosiert med lavere rikdom og mangfold av taxon, vesentlig høyere andel av tolerante enn følsomme arter, lavere ASPT indeks score, forhøyede nivåer av døgnflue-metaller, og en litt redusert pH. ASPT score viste seg å være signifikant negativt korrelert med konsentrasjoner av metaller i døgnfluer (alle testede metaller og en svært metallbelastet hovedkomponent førsteakse), som støtter bruk av ASPT indeksen som en relevant indikator og responsvariabel for metallforurensning.

Resultatene viser at vei- og tunnelbygging i områder med alunskifer presenterer bekymringer knyttet til økologisk integritet og forhøyede metallkonsentrasjoner, og tiltak bør tas for å unngå å svekke integriteten til mottagende ferskvannsøkosystemer.

List of Abbreviations

General:

ASPT	Average Score Per Taxon
EPT	Ephemeroptera, Plecoptera and Trichoptera
NMBU	Norwegian University of Life Sciences
PC1	Proxy value for first mayfly metal gradient (PC2 = second, PC3 = third)
NPRA	Norwegian Public Roads Administration
PCA	Principal Components Analysis
PC axis 1	First Principal Component
PC axis 2	Second Principal Component
PC axis 3	Third Principal Component
RDA	Redundancy Analysis
Rv4	"Riksvei 4" State Highway
WFD	Water Framework Directive

Metals/nuclides:

Al	Aluminium
As	Arsenic
Ca	Calcium
Cd	Cadmium
Co	Cobalt
Cu	Copper
Fe	Iron
Mn	Manganese
Ni	Nickel
Pb	Lead
S	Sulphur
Th	Thorium
U	Uranium
Zn	Zinc

Station codes:

13	2013
15	2015
S	Spring
A	Autumn
VU	Vigga upstream
Vøi	Vøien
Sch	School
VDS	Vigga downstream
Nor	Nordtangen
VU2	Vigga upstream 2

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1 Introduction

Transportation is, in its broadest sense, key for modern societies to function properly, and for both local and global economies be able to flourish (Europ Comm 2011). Not only does it facilitate prosperity through international cooperation and exchange of goods (called the internal market in the European Union (EU)), it also supports tourism and freedom of travel which is important both for the economy and the well-being of its people (Europ Comm 2011). The economic importance of the transport sector as a whole is also evident in that it employs a significant proportion of the global workforce (Europ Comm 2011). When incorporating all facets, the transport sector accounts for 6% and 16% of total occupational employment and 6.3% and 10% of the gross national product in the EU in 2013 and the United States in 2002, respectively (Transport Research and Innovation Portal 2013; U.S. Department of Transportation n.d.). However, such benefits do not come without considerable environmental costs. For example, the transport sector has a momentous carbon footprint (Europ Comm 2012), and there is an overwhelming scientific and political consensus that emissions must be curbed in order to prevent irreversible damage to the environment (Europ Comm 2011; Nordic Roads 2012).

Other transport-related issues and concerns that are frequently raised include local air and noise pollution as well as pervasive impacts on important natural resources, such as land and water (Angermeier et al. 2004; Europ Comm 2011). Both the construction phases and the subsequent use and management of roads (Figure 2) affect the composition, functioning, and ecological integrity of surrounding ecosystems (Angermeier et al. 2004). The degree of degradation depends on various external factors and the extent of the project at hand, but impacts of road construction commonly disrupt wetland, forest and stream ecosystems, as well as the biotic communities therein (Angermeier et al. 2004). The ecological impacts of road construction on nearby waterways – a significant but often overlooked issue – will be the focal concern for this study.

Tunnelling, together with road construction, is known to produce a plethora of both synthetic and natural contaminants, many of which become waterborne and eventually end up polluting downstream aquatic environments. These contaminants typically involve suspended particles, hydrocarbon spills and leakages, acidic and basic runoff, heavy metals, and elevated nitrogen levels (Nordic Roads 2012; Pabst et al. 2015; Vikan & Meland 2013). The contamination of surrounding water bodies is usually higher during tunnelling than during road construction (Næss 2013) due to the excessive volumes of water typically being used during its construction phases, especially drilling and cuttings removal (Meland 2016). Although tunnelling water is treated in several steps on site before being released to downstream recipients, the system is not 100% efficient and it is therefore normal that some contaminants end up leaving the site untreated (Vikan & Meland 2013).

Through its long-term research program NORWAT (Nordic Road Water), the Norwegian Public Roads Administration (NPRA) are committed to ensure that road construction is undertaken using top environmental standards, and an overarching goal is to gain the necessary knowledge to build and maintain the road network in the most sustainable and environmentally friendly fashion (Åstebøl et al. 2011; Nordic Roads 2012). This study, along with those of various MSc and PhD candidates and NPRA employees, seeks to uncover important knowledge gaps, which will help underpin key road construction decisions in the future. On expected completion in 2020, the NPRA will have invested approximately 4.3 billion NOK in the upgrading and construction of a new and more efficient road system at Riksvei 4 (Rv4) at Hadeland in the county of Oppland, Norway (Statens vegvesen 2016). The project spans 20.7 kilometres from Roa in the south to Lygna in the north (Figure 1, Figure 4).

The construction of a bypassing underground tunnel will effectively relieve traffickers from having to pass through the well-known bottleneck that currently goes through the heart of Gran (Statens vegvesen 2016). By moving heavy traffic out of urban areas, the project will also improve upon the standards of traffic safety, environmental conditions, and public health (Statens vegvesen 2016). However, based on previous experiences, it is recognized that construction activities pertinent to this construction project will likely have an ecological impact on aquatic macroinvertebrate communities inhabiting nearby streams and rivers (Åstebøl et al. 2011). Unfortunately, there are also currently large knowledge gaps as to the impacts of road construction and use on biota in Norwegian freshwater sources (Jensen et al. 2014).

Although the majority of the Rv4 tunnel will be drilled through bedrock composed of shale and limestone (Appendix 5), some parts will be drilled through alum shale (black shale) bedrock, which is reason for extra concern and precaution (Santos 2014). Alum shale is typically high in pyrite (iron-rich mineral), uranium (U), aluminium (Al) and other heavy metals (Endre & Sørmo 2015; Santos 2014), which are known to be of detriment to freshwater macroinvertebrates at certain concentrations. Of all shale types, alum shale has the highest concentrations of uranium, and some layers may contain more than 200 mg/kg (Endre & Sørmo 2015). The alum shale bedrock that will be excavated during tunnel construction is particularly uranium rich, and a nearby pit has been designated for dumping of excess material and construction-related waste (Ahmad 2015). This may cause problems as such a practice can increase mobilisation of metal contaminants, which in turn may leach into groundwater (Ahmad 2015) and surface water. As Norway is politically committed to uphold the minimum ecological requirements set by the EU Water Framework Directive (WFD), surface and groundwater pollution is a real concern that must be addressed accordingly.

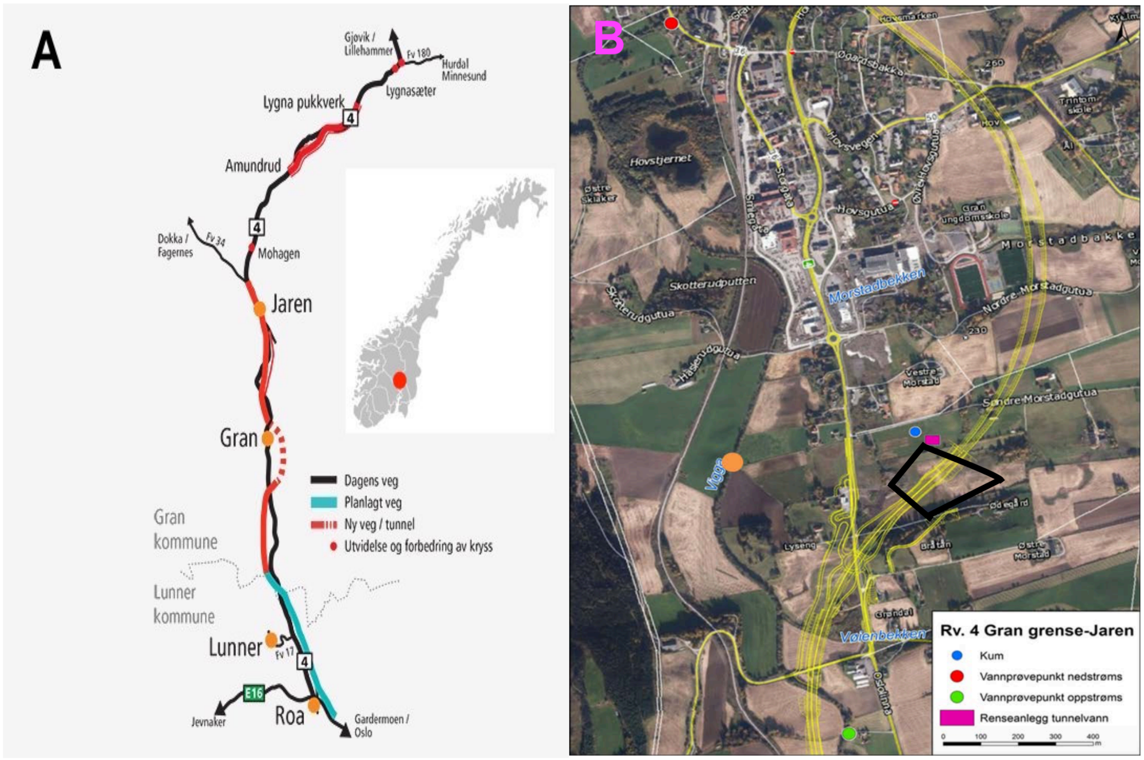

Figure 1 – **A**: Map of the proposed Rv4 road and tunnel construction work. Red = new road, dashed red = new tunnel, turquoise = planned road (south of Gran municipality), black = old road. Copied from Fjermestad (2013). **B**: Map of construction measures. Pink square = tunnel water treatment plant, orange circle = release point for treated tunnel water into Vigga, red circle = Vigga downstream station, green circle: Vigga upstream station, blue circle = manhole, black outlined area = pit section for deposition of sediments. Copied from Leikanger et al. (2015b).

The primary aim of this project is to establish if the Rv4 construction project has imposed significant ecological changes in benthic macroinvertebrate assemblages in downstream run-off areas. Macroinvertebrates are excellent bioindicators (see 2.3) and their assemblages may constitute a significant food source for various resident species, such as brown trout (*Salmo trutta*), European minnows (*Phoxinus phoxinus*) and noble crayfish (*Astacus astacus*). Changes in community structure of benthic macroinvertebrates can potentially alter local fish communities through bottom-up induced changes in available fish diets and competition regimes (Persson et al. 2014; Rustadbakken et al. 2011). In addition to being an integral constituent of all aquatic food webs, macroinvertebrates also serve important functions in the uptake and cycling of nutrients in all aquatic environments (Iwasaki et al. 2009; Zeybek et al. 2014). Consequently, sudden bottom-up changes to the make-up of macroinvertebrate communities can have several ecological implications in otherwise stable ecosystems (Persson et al. 2014), which may, in turn, negatively affect the socioeconomic and ecological values of recipient freshwater sources.

1.1 Main objective

i) To assess if the Rv4 construction project has imposed any negative ecological effects on benthic macroinvertebrate communities in streams and brooks inside impacted areas.

   a. Which environmental variables are the most important drivers for the observed variation in macroinvertebrate assemblages?

   b. Are levels of mayfly metal concentrations elevated in impacted areas compared to reference areas? If so, establish whether potential increases in mayfly metal concentrations are mainly due to anthropogenic (road construction) or natural (seasonal, annual) processes, and if such increases have negatively affected benthic macroinvertebrates.

2 Theory

2.1 Road construction and pollution

It is safe to say that a constantly developing world takes a substantial toll on the environment. Especially prone to deterioration are aquatic environments, and the biota therein are exposed to the deleterious effects of multiple external stressors, such as metals, fine inorganic particles, organic compounds, toxins, salts and other nutrients (Persson et al. 2014). Road and tunnel construction can be considered a major culprit in this regard.

Due to various chemical properties and environmental issues associated with excavation of bedrock and sediment material, the development and construction of transport routes, such as roads, highways and railways, can potentially be a pervasive and nasty affair (Wheeler et al. 2005).

Once a tunnel is operational, maintenance-related issues mainly revolve around the downstream release of often untreated tunnel wash water (NFF 2009). The construction phase, however, poses a wider range of problems. Construction-related environmental concerns commonly include the release of nitrogenous compounds from explosives, increased pH stemming from large volumes of cement, petrochemical and other chemical spills from equipment and machinery, and runoff from grouting work and diffuse sources (NFF 2009). Alum shale, which has the greatest acid-forming potential of all shale types, is often associated with added expenses, increased environmental risks, damage to buildings and equipment (from low pH), and special requirements for disposal and landfills (Endre & Sørmo 2015). However, in their inert and untouched states there is usually no reason for concern, and it is rather their weathering potential during disturbance (i.e. bedrock excavation) that determines their ability to become problematic (Endre & Sørmo 2015)

In addition, bedrock excavation alone presents a host of physicochemical issues. A review study by Pabst et al. (2015) and additional references summarise the general ecotoxicological effects that excavation of bedrock may have on surrounding aquatic environments (2.1.1 - 2.1.3). The three main effects are elevated levels of dissolved particles, the production of acids and subsequent mobilisation of metals, and the release of detrimental radionuclides.

2.1.1 Particle loading

Most major features of road construction, such as drilling, rock blasting, crushing and digging, give rise to a plethora of mineral particles of different shapes and sizes (typically ranges from a few micrometres to more than 60 centimetres) (NFF 2009). Particle sizes and sedimentation speeds are usually positively correlated, and the sedimentation process goes slower in freshwater than in saltwater due to a general lack of ions. The finest sediments therefore reach the furthest downstream. Depending on particle size, these fine sediments can be detrimental to biota (Persson et al. 2014), especially those of hard composition and with sharp edges – two characteristics that are often incompatible with sensitive biological tissues, such as gills and eyes, and may directly damage the fish (NFF 2009; Price 2013) or cause ulcers to form. It is therefore key that proper management measures are taken in order to minimise contaminated runoff (Næss 2013).

2.1.2 Acid production and heavy metal mobilisation

The elemental composition of rocks and bedrock dictates their chemical and physical properties. It is well established that a lowering of pH may significantly affect the weathering properties of different rocks, and that this in turn increases the solubility of various mineral elements (Price 2013; Santos 2014). For example, when bedrock minerals containing high levels of sulphide and other sulphur-compounds are excavated and make contact with water and oxygen, sulphuric acid is produced and the potential for heavy metal and radionuclide mobilisation increases (Endre & Sørmo 2015; Hjulstad 2015; Santos 2014). One can assume that the lower the runoff pH, the higher the concentration of dissolved toxic heavy metals, such as cadmium (Cd), arsenic (As), lead (Pb) and nickel (Ni). A significant issue in systems affected by low pH runoff (especially below 4) is the mobilisation of such metals, as well as aluminium (Al) and iron (Fe) (Endre & Sørmo 2015), which all have, at a certain concentration, the potential to harm sensitive organisms in the recipients. An additional issue associated with low pH solutions is that the ionic form of these toxic elements become more prevalent than their complex or colloidal forms. Acid mine drainage from sulphur-rich rocks is typically characterised by high acidity (Hjulstad 2015) and high concentrations of dissolved metals, which has often been the root cause for several water contamination episodes in mining districts the world over (Price 2013).

2.1.3 Radionuclides

Sediments and bedrock that include radioactive minerals may pose a threat to biota. This is especially true for alum shale, but rocks like syenite, pegmatite and granite are also problematic for construction in Norway due to their high levels of uranium and thorium

(Th). If these radioactive minerals are unstable they can decay in the environment into the radioactive elements radium (Ra) and radon (Rn) (Endre & Sørmo 2015), which may reach levels where they become detrimental to biota. For example, radioactivity limits stipulated by the Norwegian Radiation Protection Authority are often exceeded in alum shale runoff. Although not much is known about the potential "cocktail effects" of these issues, nor their environmental interactions with other pollutants, there are concerns that the combined effect may outweigh the sum of their individual effects. However, much research remains to be done before any conclusions can be drawn.

For the aforementioned reasons, it is critical that proper geological investigations and surveys are undertaken prior to construction with the aim to identify the contaminating potential of the bedrock materials. Bedrock composition varies greatly in Norway as a result of different geological processes (Endre & Sørmo 2015), so these investigations are necessarily site-specific. Secondly, measures to dispose of construction waste and contaminated runoff must be in place so that the impact on the recipients is minimised. For example, sedimentation pools and silt curtains in lakes are common methods for reducing the impact of larger particles, whilst various chemical settling agents can be used to precipitate smaller particles out of solution (Pabst et al. 2015). An efficient way to reduce the impacts of erosion and runoff on watercourses is to implement riparian zones/vegetation buffer strips into landscape planning along the watercourse. To prevent or minimise acidic runoff from forming in the first place, wastewater containing acid-forming sulphur minerals may be removed before it is properly oxidised, which in turn will minimise the mobilisation of heavy metals (Pabst et al. 2015). Taking the necessary on-site steps to prevent or restrict dispersal of contaminants can effectively reduce the need for long-term water treatment (Pabst et al. 2015).

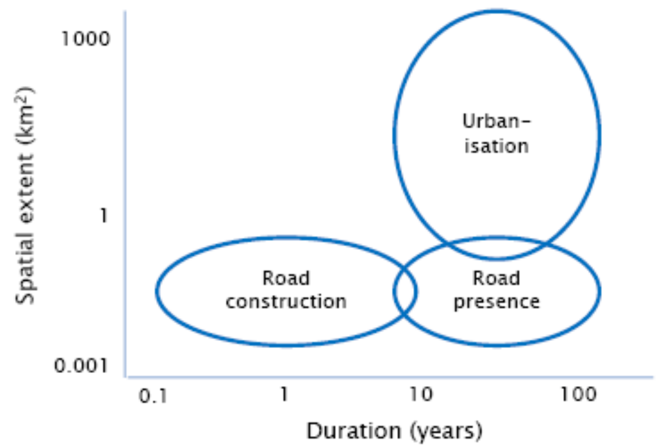

Figure 2 – Spatial and temporal extent of various aspects of road development (logarithmic axes/scale). Copied from Meland (2016) who modified from Angermeier et al. (2004).

2.2 EU Water Framework Directive

The WFD is a water policy framework adopted by the European Union (EU) in 2000 as part of the European Water Policy, and which overarching aim is to standardize the management of water bodies (fresh-, ground- and coastal water) throughout Europe, particularly in terms of protecting environmental integrity, ecological status, and ensuring sustainable use (Europ Comm 2016; Miccoli et al. 2013; Miljødirektoratet 2013; Rustadbakken et al. 2011; Vannportalen 2015). The main goal across all EU member states is for all water bodies to achieve and/or maintain at the very minimum a "good status" (Figure 3). Although Norway is not a member country of the EU, it is committed to implementing the WFD within the constructs of Norwegian legislation (Vannportalen 2015). A primary goal of this commitment is developing regional or national water management plans that are both comprehensive and ecosystem-based (Miljødirektoratet 2013). In order to assess the ecological state of water bodies, classification must be based first and foremost on biological variables (i.e. macroinvertebrates) since they reflect important ecological characteristics such as productivity and species richness (Johnson et al. 2006; Miljødirektoratet 2013). Although less common, other important organisms used in bioassessment include certain fish species, macrophytes, benthic algae (i.e.

diatoms) and macrophytes, and using multiple bioindicators in ecological assessments may be advantageous as it arguably detects ecological changes more accurately (Carlisle et al. 2008; Johnson et al. 2006; Knoben et al. 1995). Measuring physicochemical variables often comes second or in addition to biological variables in bioassessments, with the exception of groundwater, which is exclusively based on physicochemistry (Johnson et al. 2006; Miljødirektoratet 2013).

2.3 Benthic macroinvertebrates and biotic indices

The unidirectional flow and dynamic nature of rivers and streams can make assessing its ecological health a challenging task. Where methods that solely rely on chemical indicators may fail to reflect a recent contamination event, biological indicators (bioindicators) can fill in the missing information gaps. Benthic macroinvertebrates are excellent bioindicators of stream water quality due to various factors, such as their relative abundance, manageable size, and ease and cost-efficiency of sampling (Blijswijk et al. 2004; Chiba et al. 2011; Duran 2006; Reynoldson & Metcalfe-Smith 1992; Santoro et al. 2009). Their community structure is also known to respond to temporal changes in water quality (Clements 1994; Persson et al. 2014), and the life history and pollution-responses of several species are known (Persson et al. 2014; Reynoldson & Metcalfe-Smith 1992). Although using fish communities as bioindicators carry some advantages (e.g. long-lived), they have displayed pollution avoidance behaviour and the migration patterns of some species may raise methodological concerns (Knoben et al. 1995). The relative inability of macroinvertebrates to relocate far following a contamination event (e.g. an upstream chemical spill) and the fact that communities are composed of several different faunal orders, renders them a good biological assessment tool in both space and time (Knoben et al. 1995). In fact, a review of 100 different biological assessment methods found two thirds to be macroinvertebrate-based (Knoben et al. 1995). In order to get a good picture of benthic macroinvertebrate assemblages in an area, sampling should occur at minimum twice a year; around two weeks after the spring flood and during autumn around October/November (Miljødirektoratet 2013; Rustadbakken et al. 2011).

The knowledge gained from the increased use of bioassessment indices, such as the Biological Monitoring Working Party (BMWP) and its derivative index Average Score Per Taxon (ASPT), has positively influenced surface water policies and management plans all over Europe for the past few decades (Metcalfe 1989). It has also been used elsewhere, such as in South Africa, where it has provided a means for ecological comparisons against reference rivers (Bellingan et al. 2015). The ASPT score is a common evaluation method used to indicate the ecological state of, for example, wadeable streams, and is based on the presence or absence of benthic macroinvertebrate taxa (Miljødirektoratet 2013; Zeybek et al. 2014). The majority of these are families of EPT orders – Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). Like most other biotic indices, the ASPT index is a numeric score system based on the specific sensitivity of different macroinvertebrate families to pollution. Each family has a specific sensitivity score, ranked from 1 (very poor, species are very tolerant) to 10 (very good, species are very sensitive), and each new family is counted only once (Miljødirektoratet 2013; Zeybek et al. 2014). The BMWP score is the sum of these individual family scores, and the ASPT score is then derived by calculating the average (Miljødirektoratet 2013).

Although commonly used to estimate the degree of eutrophication and organic enrichment in water bodies – especially the EPTs (Clements 1994) – benthic macroinvertebrates and their respective ASPT scores are frequently used as a surrogate measure of water quality and ecological change, and especially within the scope of the EU Water Framework Directive (Zeybek et al. 2014). As the composition of macroinvertebrate communities and their family-level sensitivity to pollution may vary from region to region, biotic indices should as accurately as possible reflect the species present in the region of which sampling will take place (Roche et al. 2010). For example, a Brazilian study of a coastal river ecosystem concluded that four of the most common macroinvertebrate-based indices, which are mainly based on European macroinvertebrate fauna, had limited applicability, and all four were in fact shown to be inferior to physicochemical variables (Gonçalves & de Menezes 2011). However, Miljødirektoratet (2013) has provided an ASPT index specifically for Norwegian freshwater taxa, which was used as the basis for assessing the ecological state of the sample stations in this study (Appendix 3).

Very Good		Acceptable ecological condition
Good		
Moderate		Action is necessary to achieve acceptable ecological status
Poor		
Bad		

Figure 3 – The five ecological quality classes (QCs), defined by the EU Water Framework Directive (WFD) and applicable to European aquatic ecosystems. Only classes "Good" and "Very Good" are acceptable, lower ecological standings must be restored to acceptable levels.

2.4 Ordination analyses and multivariate statistics

When undertaking research within the realm of community and landscape ecology, one may come up against large variations in species richness and evenness across a multitude of different stations and environmental gradients. Making sense of such potentially complex datasets, and the different variables and variation therein, may be a cumbersome process. Depending on the multivariate nature of the data, attempts to find the most significant explanatory factors by looking at each variable separately makes little statistical sense. In these cases, the best analytical tools will be those that account for the multidimensionality of the data in as few tests as possible, which also reduces the chance of Type I errors (false positives). Enters ordination (from *ordinare* – "put in order"), an increasingly common multivariate statistical approach for ecologists wanting to investigate the continuity of change in the composition of biotic communities. The ordination analyses for this thesis were undertaken in Canoco 5 (Smilauer & Leps 2014), which employs various ordination techniques in order to identify and describe the trends in the response (input) data.

Canoco 5 divides these techniques into two umbrella analyses – unconstrained ordination and constrained ordination (Table 1). Methods of unconstrained ordination typically involves Principal Components Analysis (PCA) or Correspondence Analysis (CA).

Basically, these assess data with multiple response variables (i.e. biological species) with the aim to identify the axes that are the most instrumental in shaping the observed structure in the response data (i.e. species composition). Constrained ordination, however, is introduced where there are one or more accompanying explanatory (predictor) variables (i.e. environmental variables) that can be used to explain the variation in the response data. The two most common constrained ordination methods are the redundancy analysis (RDA) and the canonical correspondence analysis (CCA).

Table 1 – Ordination analysis options, based on the desired analysis and shape of the response data curve.

	Linear	**Unimodal**
Unconstrained	Principal Components Analysis (PCA)	Correspondence Analysis (CA)
Constrained	Redundancy Analysis (RDA)	Canonical Correspondence Analysis (CCA)

Whether to choose a linear or the unimodal ordination model depends on the amount of turnover (SD) units (gradient) of the response data, and an automatic background test in Canoco 5 suggests which model is most appropriate. Due to the linearity of the data, only PCA and RDA was used for the ordination in this study. The eigenvalues of the different axes (Axis 1, Axis 2, Axis 3 etc.) represent the variation in the data; the higher the eigenvalue of an axis, the more variation in the data is explained by the variable(s) that that particular axis represents. In an ordination diagram, the relative distribution of cases and direction of arrows (response data) signifies their correlation. For example, arrows going in opposite direction are negatively correlated, which, in the case of this particular study, is indicative of opposing environmental requirements. The same interpretation applies to cases; the further away from each other, the fewer environmental and ecological attributes they have in common, and vice versa. The longer the arrow, the more important that particular response data is.

3 Materials & Methods

3.1 Study area

The study area is located in and around the town of Gran in Oppland County (Figure 1, Figure 4). Sampling locations (herein referred to as stations) were mainly located in streams and brooks that eventually empty into Lake Jarenvannet at its southernmost tip. As seen in Table 2 and Figure 4, the four original stations sampled in both 2013 and 2015 were Vigga downstream (i.e. downstream of release point), Vigga upstream, Vøyen and School. Nordtangen, a smaller brook running into Jarenvannet from the east, was included in the spring field session. However, these replicates unfortunately became invalid due to tagging issues, and, in addition, some other replicates from other stations went missing from 2013 (see raw data, Appendix 6). By spring 2015, general construction work in the area, which was not directly related to the tunnel construction, had likely affected Vigga upstream. Therefore, Vigga upstream 2, 8.4 kilometres upstream from the river mouth and 5.6 kilometres upstream from Vigga upstream, was included as a new reference station in 2015.

Table 2 – Overview of the six sampling stations, and their statuses in 2013 and 2015 in respect to reference or impact. School and Nordtangen were not sampled during autumn 2013, and all replicates from the latter were lost in spring 2013 due to tagging issues. Vigga upstream 2 was first introduced as a new reference station in 2015 when it was realised that Vigga upstream would have been affected by general construction-related pollution.

			2013		**2015**	
Station #	**Name**	**Name abr.**	**Spring (Jun 25)**	**Autumn (Sep 18)**	**Spring (June 19)**	**Autumn (Oct 8)**
2	Vigga upstream	VU	Reference	Reference	Impact	Impact
3	Vøien (brook)	Vøi	Reference	Reference	Reference	Reference
4	School (brook)	Sch	Reference		Reference	Reference
6	Vigga downstream	VDS	Reference	Reference	Impact	Impact
7	Nordtangen (brook)	Nor	NA (lost)		Impact	Impact
10	Vigga upstream 2	VU2			Reference	Reference

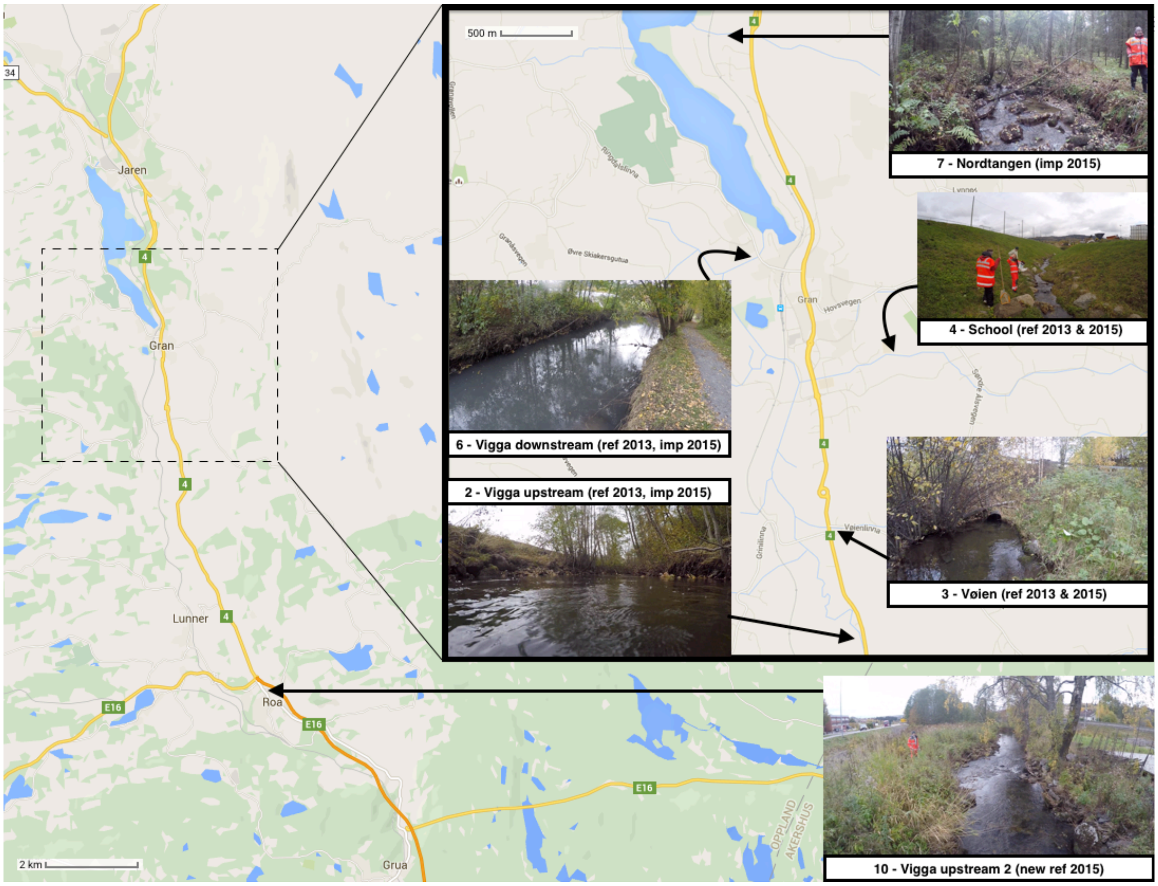

Figure 4 – The location of Lake Jarenvannet and all stations relative to Gran (60.359905, 10.572925). Vigga runs northwards into Lake Jarenvannet at its southernmost end (source: Google Maps). Ref = reference, imp = impact. See Table 2 for more information.

First round of sampling took place on June 25 and September 18 in 2013 – shortly before construction commenced – and second round on June 19 and October 8 in 2015. All samples from 2013 served as experimental controls (reference) with the underpinning assumption that these communities represent the normal ecological state of the area before any drilling or construction took place. Fast forward to 2015 and three impact stations – Vigga downstream, Vigga upstream and Nordtangen – may have been affected by two year's worth of construction-related pollution. Vøien, School and Vigga upstream 2 acted as reference stations as they were located upstream and far enough away from any construction areas or transport routes to be significantly affected by waterborne or airborne contaminants.

3.2 Collection of samples

It is important to note that the spring samples in this study were technically not sampled during spring, but instead in June (early summer). The late sampling was in 2013 due to longer periods of heavy rain and flooding, which rendered spring field work a risky undertaking. In 2015 spring weather was more gentle, however, it was decided to sample at the same time of the year as in 2013 in order to maintain continuity in the data. This nominal mistake was unfortunately discovered too late to change with regards to all ordination figures.

At each station, a standard stream kick-sampling procedure (semi-quantitative) was employed in order to get a good and cost-effective representation of benthic macroinvertebrate communities present (Norwegian Standard NS-ISO 7828 and in accordance with, for example, the stream sampling protocols outlined in Stark et al. (2001)).

Each station was divided into three replicates, whereas each replicate was collected by 3 * 20 seconds kick-sampling: holding a 450 µm mesh hand net against the current and walking along a transect on the bottom of the stream while kicking the substrate. Once the one-minute mark had passed, all the contents collected in the net were emptied into an appropriately sized tray, with larger objects such as rocks, vegetation and leaves being removed from the sample. The tray was filled approximately a third full with water from the top layer of stream, and the contents were stirred and shaken in order to oust and suspend any macroinvertebrates. The water, and everything floating in it, was then poured back into the net, and this "rinsing" step occurred enough times for one to be left with primarily abiotic material such as rocks, gravel and other sediments. This material was dumped back into the stream, and all the contents of the net were preserved in a labelled container or plastic bag containing 96% ethanol.

3.3 Sample processing

The contents of each individual replicate were homogenously distributed in a squared tray and divided into four equal-sized subsamples. One subsample was transferred into a second tray for sorting, whilst the remains in the original tray (mother sample) were inundated with ethanol and then covered to avoid evaporation. Macroinvertebrates were systematically picked out of the subsample by repeatedly adding water to the tray and pouring into a petri dish for closer inspection under a stereo or dissecting microscope. Specimens from each replicate were contained in a smaller labelled glass vial, prefilled with 96% ethanol. When all macroinvertebrates had been transferred, the remains in the mother sample was examined with the naked eye for about five minutes in order to find any species that were potentially absent or overlooked in the subsample. Any species found in the mother sample were transferred to a second glass vial. The exact procedures for kick-sampling and sample processing are demonstrated in the following instructional video: http://bit.ly/1QdRiom.

Two vials were therefore prepared per replicate; one with the specimens from the subsample and one with those from the mother sample. It is also critical that the abundance of each individual species in each subsample is multiplied by four, in order to arrive at the total sample size for each replicate (keep in mind, semi-quantitative estimate)). Following completion of macroinvertebrate sorting, taxonomic identification commenced. The literature that was used for this purpose included Lillehammer (1988), Wallace et al. (1990), Aagaard and Dolmen (1996), Dobson et al. (2012), Nilsson (1996), (Nilsson 1997) and Edington and Hildrew (1995).

The benthic macroinvertebrates that were collected consisted of a variety of organisms at different taxonomic levels. The taxonomic level to which all of these individuals (~42,000) needed to be identified was largely determined by the available knowledge and literature about them, their specific role as bioindicators, and the relative difficulty of identifying them. For example, certain organisms, such as the oligochaetes (worms) and the araneaes (spiders), were only identified to their respective classes, whilst it was sufficient for the scope of this study to determine coleoptera (beetles) and diptera (true flies) to family level. Other organisms, such as the EPTs – Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) – were to the best ability identified to species.

Due to organismal disintegration and degeneration (i.e. damage from sampling and handling), various individuals were either somewhat or almost completely indeterminable, and were those cases identified down to the lowest possible taxonomic level. Where relevant, these are denoted with –W at the end of the taxa codes (Appendix 1) used in the ordination analyses. Those that were supposed to be determined to species, but no further than genus was possible, are denoted with –Sp. For the sake of simplicity, all taxonomic levels (regardless of the possibility of taxonomic overlap) are referred to as taxa in the subsequent ordination analyses and in the text, but those that are especially relevant will be discussed to further detail.

3.4 Statistical analysis

The statistical analyses presented in this report were constructed using a software combination of R (R Development Core Team 2011) and Canoco 5 (Smilauer & Leps 2014). Microsoft Excel 2016 was also used to present some of the results. All statistical tests are based on significance level alpha = 0.05.

For statistical purposes, the original species dataset was slightly modified in order to avoid making the subsequent analyses unnecessarily complex: a few species occurred at two or more stages of their respective life cycles, typically as larvae (L), pupae (P) and adults (A). In all such instances, all three life stages were combined for each species and recorded as just one number. The original dataset (raw data) in its entirety is provided in Appendix 6.

The response data used in this study was primarily the species data, but *in situ* mayfly metal concentrations were also used. Two main groups of predictors were used to explain the response data (

Table 3): 1) Effects included station, season, year and treatment, and 2) covariates for use in constrained ordination analyses were *in situ* mayfly metal concentrations and physicochemical water quality variables (pH, temperature and conductivity). Water metal concentrations were also measured and collected for use in analysis, but were omitted from statistical analyses as it was concluded that mayflies would more precisely reflect temporal fluctuations in metal concentrations for each station (see PCA of water metals and associated analysis in Appendix 9). Another issue with using water metals as covariates for the species data was that not all stations were sampled the same day as the kick-sampling took place, whereas the mayflies were collected *in situ* and concurrently.

3.4.1 Ordination

Species and metal response data were all log-transformed and centred (metals were standardized in addition). For constrained analyses, numeric predictor values were first log-transformed, with the exception of pH, which is an already log-transformed value. Canoco 5 automatically centres and standardizes all explanatory variables (Smilauer & Leps 2014). In order to reduce the amount of metal covariates, a PCA of 14 metals (based on station averages) was executed (Figure 13) and the case scores were extracted as PC1 and PC2 (Table 9). These two new measurements were then used as proxy values for metal concentrations in constrained analyses (in reality PC axis 1 and PC axis 2 of the metal data, respectively). In addition, five metals – Al, Cd, Fe, U and Th – were used as independent covariates due to their association with alum shale bedrock. For the sake of clarity, only 30 of 85 taxa are displayed in all figures according to their relative fitness/weight (full PCA in Appendix 2).

For unconstrained ordination analyses, all datasets were used in their entirety and all replicates were included. These complete datasets were compared to their corresponding explanatory variables (station, season, year and treatment). For all constrained analyses, both complete datasets (including station replicates) and averaged datasets (average of station replicates) were used; the former when only effects acted as predictors, and averaged data when effects and/or covariates acted as predictors (station averages had to be calculated for constrained coupling with covariates in order to avoid ending up with erroneous pseudo-replicates). In all constrained analyses where the importance of covariates was tested, season, treatment and year were included as correction effects as they reflected the sampling structure and the objectives (except for Figure 15 when adding year as effect created too many degrees of freedom). Station was found to have very little prediction power, and was therefore omitted as a correction effect in these cases. When the most important predictors were identified, six 3-group variation partitioning tests were executed in order to identify the three predictors that had the greatest individual impact on species distribution.

Table 3 – Overview of all predictors (effects and covariates) for use in constrained analyses.

Effects	
Station	Vigga upstream, Vøien, School, Vigga downstream, Nordtangen, Vigga upstream 2
Season	Spring 13, Autumn 13, Spring 15, Autumn 15
Year	2013, 2015
Treatment	Reference (control), impact (impacted areas)
Covariates	
Aluminium (Al)	Key nuclide associated with alum/black shale bedrock
Iron (Fe)	Key nuclide associated with alum/black shale bedrock
Cadmium (Cd)	Key nuclide associated with alum/black shale bedrock
Thorium (Th)	Key nuclide associated with alum/black shale bedrock
Uranium (U)	Key nuclide associated with alum/black shale bedrock
pH	Important in mobilisation of metals from bedrock
Conductivity	
Temp °C	
PC1	Represents first metal gradient for use in constrained ordination
PC2	Represents second metal gradient for use in constrained ordination
ASPT	Average Score Per Taxon (based on the BMWP Index)

3.4.2 Univariate analyses

In order to quantify and test for treatment effects and effects from various environmental variables on univariate response variables (i.e., ASPT and diversity indices), linear mixed effect (LME, e.g., Zuur et al. (2009)) models were used. In these tests, replicate samples nested under station, were used as *a priori* random effects in all candidate models (to account for within-station variation). For the fixed part of the LME model structure, "season" and "treatment" were always included as the former has repeatedly been demonstrated to affect stream benthic invertebrate community structure (e.g. Persson et al. (2014)), and the latter because quantification of potential treatment effects was the main objective of this study. LMEs were fitted using the "lmer" function available from the lme4 library in R (Bates et al. 2015), and model selection among candidate models were conducted by means of the Akaike's Information Criterion, AIC (Anderson 2008; Burnham & Anderson 2002). AIC is estimated as the sum of a fitted model's deviance (i.e., residuals if fitted by ordinary least square regression) and two times the number of parameters (K) included in the model (AIC=deviance + 2*K). The rationale behind this information criterion is to seek models that most efficiently balance parameter estimation precision and bias. Among a set of candidate models fitted to the same response data set, the model with the *lowest* AIC-value is selected as this model has the highest AIC support among the candidates. All other models are ranked relatively to this selected model according to the difference in AIC-value (ΔAIC). In addition to the ΔAIC, the relative likelihood for a model among candidate models can be estimated as exp(-0.5 * ΔAIC), which is often framed the AIC weight (Burnham & Anderson 2002). In this study, I used a corrected version of the AIC (AICc) that penalize complex models to a larger degree when n is small: AICc = deviance + 2K*(n/(n–K–1)) (Burnham & Anderson 2002).

For estimation of LME R^2-values (i.e., explained variance), both marginal R^2 (variance explained by fixed factors) and conditional R^2 (variance explained by fixed and random factors) were estimated using the r.squaredGLMM function in the MuMIn library (Nakagawa & Schielzeth 2013).

Professor Thrond Haugen was very involved in this part of the statistics (using R) and I can thank him for creating figures 9, 11, 19 and 20, and tables 5, 6, 7 and 8.

4 Results

4.1 General trends in macroinvertebrate assemblages

A total of 85 taxa were identified from all six stations, consisting of organisms identified to taxonomic levels ranging from class to species (Figure 5). The vast majority of these, roughly 87%, were members of EPT orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies), as well as Coleoptera (beetles), Oligochaeta (worms) and the family Chironomidae (non-biting midges). The remaining 13% consisted of a mix of, amongst others, Diptera (true flies), Aranea (spiders), Hydrachnidiae (water mites) and snails. Virtually all EPTs, chironomids and diptera were present as larvae, however some pupae did occur. Coleoptera were mainly present as adults, but there were some larvae as well (mainly Elmidae, no Hydraenidae).

Vøien, with 1273 individuals on average across all seasons and both years, had undoubtedly the highest abundance of all stations (semi-quantitative). This was even 61% and 68% more than Vigga upstream and School, which had the second and third highest abundance, respectively. In Nordtangen during spring 2015 there were virtually no Ephemeroptera, Trichoptera or Coleoptera present, whilst Chironomidae and Plecoptera were abundant. However, most individuals belonged to other groups of organisms. With 387 individuals on average, Nordtangen also had the lowest abundance of all stations. Vigga downstream followed closely with 414 individuals.

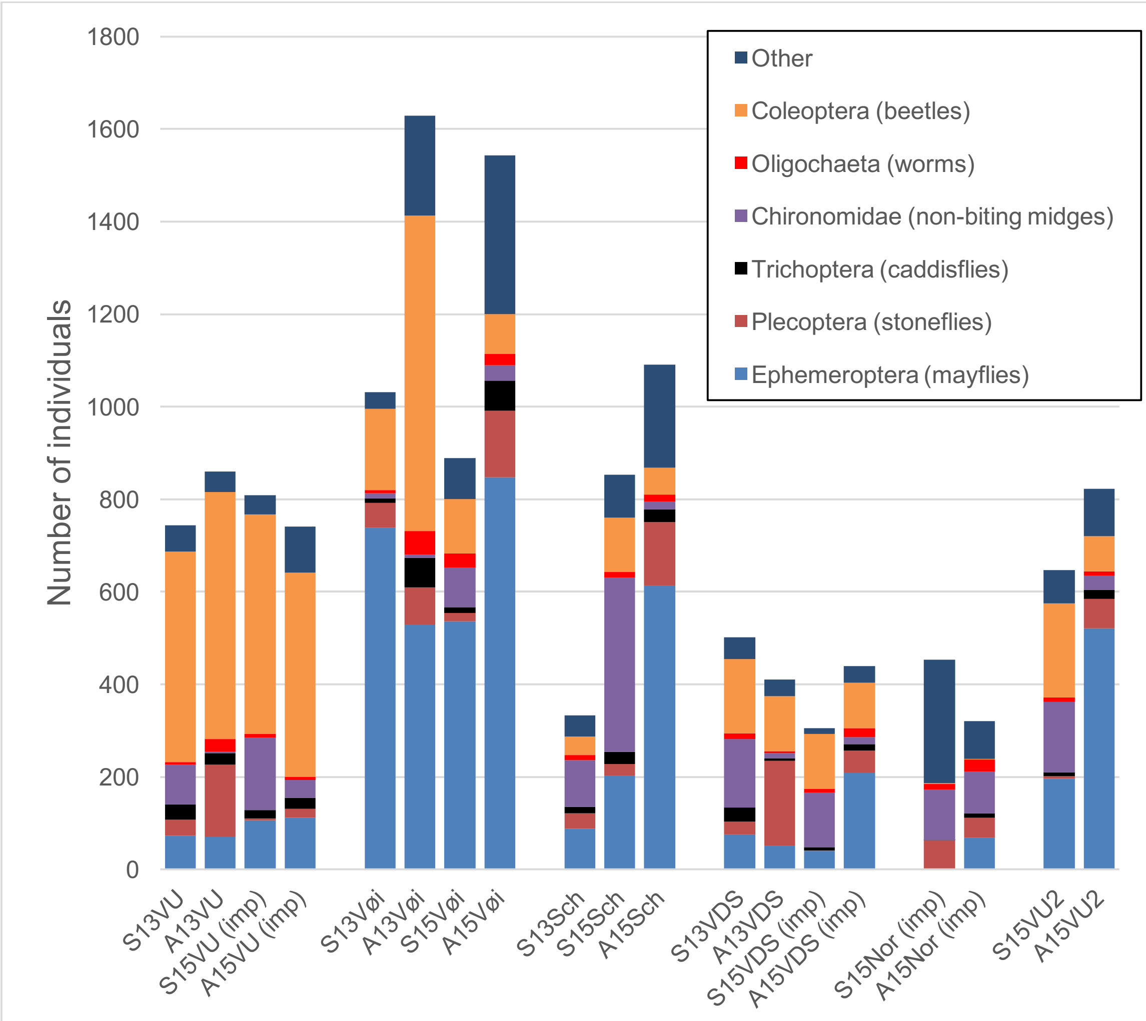

Figure 5 – The number of individuals (station averages) and composition of main groups of benthic macroinvertebrates. S = spring, A = autumn, 13 = 2013, 15 = 2015, VU = Vigga upstream, Vøi = Vøien, Sch = school, VDS = Vigga downstream, Nor = Nordtangen, VU2 = Vigga upstream 2.

Ephemeroptera made up 35.8% of total macroinvertebrate abundance, and the order was dominated by the species *Baetis rhodani*, *Alainites muticus* (formerly *Baetis muticus*) and *Baetis nigris* (99.1%) (Table 4). *B. rhodani* alone made up as much as 29.8% of total abundance. Plecoptera was considerably less prevalent (7.9% of total abundance), with the three species *Taeniopteryx nebulosa*, *Protonemura meyeri* and *Amphinemura standfussi* being the dominant species and almost equally numerous. These three species made up 41.8% of all Plecoptera. Of the EPTs, Trichoptera was the least common. Here, *Rhyacophila nubila*, *Silo pallipes* and *Chaetopteryx villosa* made up 69.3% of all species (the second most numerous taxa of Trichoptera was actually indeterminate members of the family Limnephilidae). With 27.5% of total abundance, Coleoptera was the second largest group of macroinvertebrates, and dominated by the families Elmidae (riffle beetles) and Hydraenidae (minute moss beetles) (99.7%). Families Simuliidae (black flies) and Psychodidae (drain flies), and the genus Dicranota (Family Pediciidae, hairy-eyed craneflies), dominated the Others-group (75.1%).

Table 4 – Abundances and proportions (%) for each major macroinvertebrate groups and their respective most dominant species/families (total and relative). When possible, EPTs were identified down to species level (italic), the rest to family or other taxonomic levels (not in italic).

Group	n	Total prop (%)	Species or family	n	Relative prop (%)	Total prop (%)
Emphemeroptera	15006	35.78	*Baetis rhodani*	12512	83.37	29.83
			Alainites muticus	1372	9.14	3.27
			Baetis nigris	991	6.60	2.36
Plecoptera	3321	7.91	*Taeniopteryx nebulosa*	496	14.93	1.18
			Protonemura meyeri	449	13.52	1.07
			Amphinemura standfussi	444	13.36	1.05
Trichoptera	1175	2.80	*Rhyacophila nubila*	652	55.48	1.55
			(Limnephilidae indet)	198	16.85	0.47
			Silo pallipes	92	7.82	0.21
			Chaetopteryx villosa	70	5.95	0.16
Coleoptera	11511	27.45	Elmidae	6337	55.05	15.11
			Hydraenidae	5145	44.69	12.26
Others	5689	13.56	Simuliidae	1902	33.43	4.54
			Psychodidae	1789	31.45	4.27
			Pediciidae (*Dicranota* spp)	581	10.21	1.39
			Total	**33030**		**78.77**
Chironomidae	4357	10.39				
Oligochaeta	873	2.08				
Total	**41932**	**100**				

There is quite a bit of variation in the taxa composition (Figure 6). With 20.74% first axis explains only 3.58% more than second axis, but the majority of taxa are clustered just below the first axis. In addition to this main cluster there appears to be four other smaller clusters, however, these are more diffuse and more indistinct. The occurrence of certain groups of macroinvertebrates invertebrates are completely negatively correlated, such as the family Elmidae and the genus *Dicranota*, meaning that when one is prevalent in an environment the other is likely scarce. The same applies to the chironomids and the caddisfly *Rhyacophila nubila*.

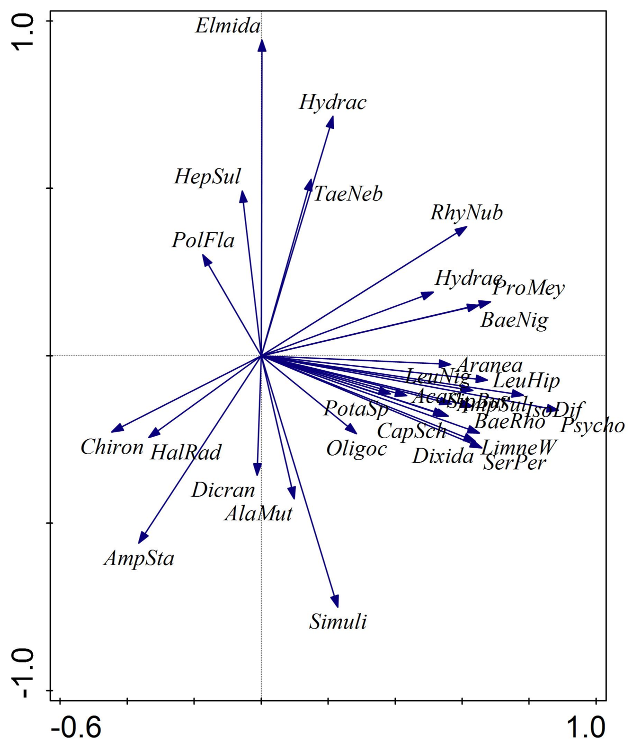

Figure 6 – PCA of total species data. For the sake of clarity, 30 of 85 taxa are displayed in all figures according to their relative fitness/weight. Response data gradient of 2.6 SD units favours linear method. No predictor variables are included in this analysis. First axis explains 20.74% of the variation in the data whilst second and third axis (not visible) explains 17.16% and 9.61%, respectively. Combined the two first axes explain 37.90% of the total variation. Taxa close to each other share similar environmental requirements. A complete PCA with all 85 taxa can be found in Appendix 2. For the full names of the different species, see Appendix 1. For associated distribution of stations and Shannon-Wiener species diversity, see Figure 7.

The different stations and respective replicates (both herein referred to as cases) cluster in different areas, however, with some overlap (Figure 7A). All Nordtangen cases (impacted) are located in the third quadrant, whilst the remaining stations are more spread out across the two main axes. The macroinvertebrate community makeup is very similar amongst the three Vigga stations, with all cases being almost exclusively located in the upper half of the graph, regardless of treatment levels and seasonal variation.

As seen in the Shannon-Wiener (SW) diversity index (Figure 7B), a greater species diversity is found in the three Vigga stations than in the three other stations. The lowest diversity is found in Nordtangen, whilst it is more even between Vøien and School. When comparing against Figure 7A one can see that most of the cases that are associated with low diversity were sampled in the spring of 2015, especially Nordtangen and Vigga downstream, whilst the difference in diversity between the remaining seasons is less distinct.

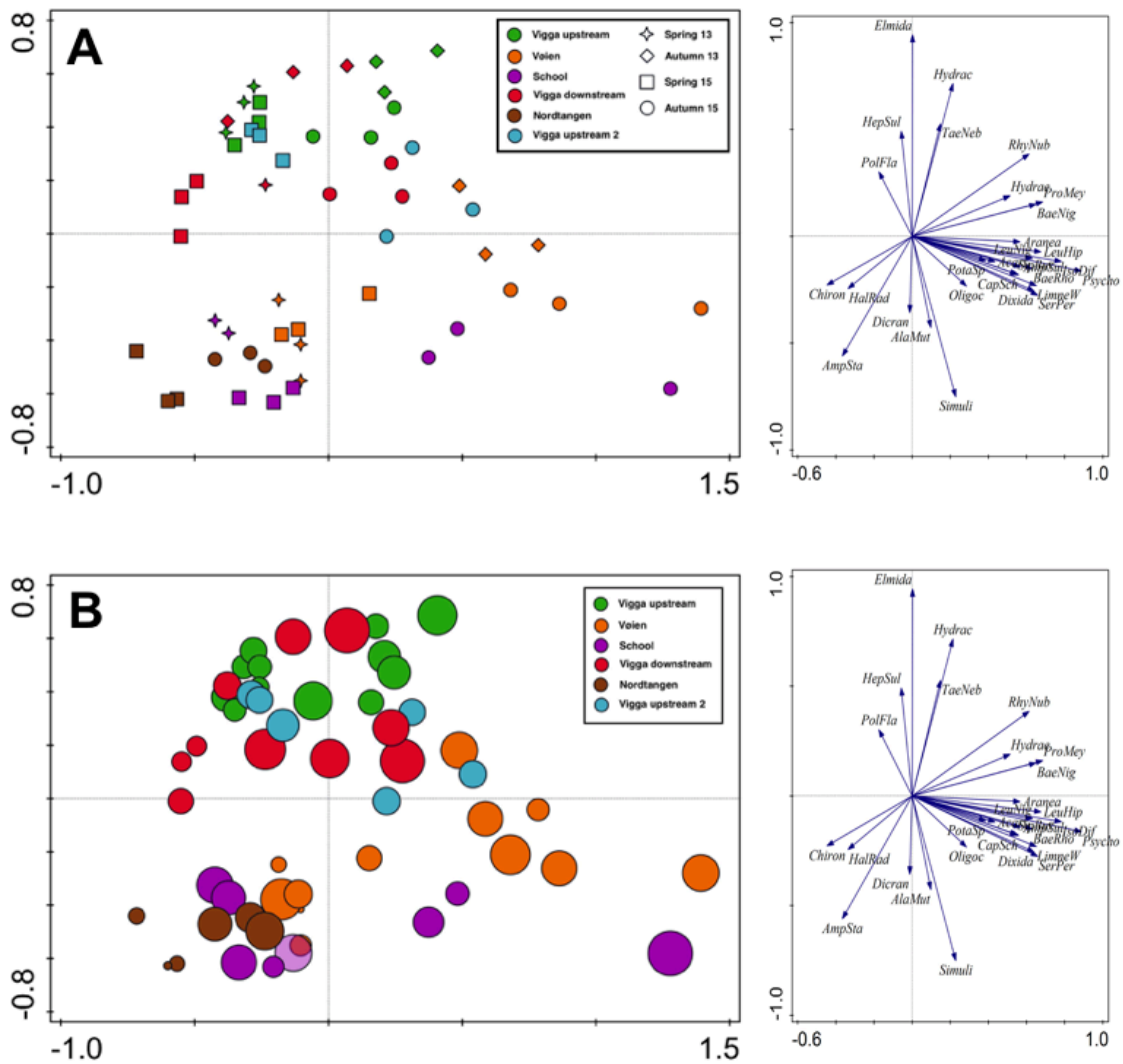

Figure 7 – **A**: Relative distribution of all stations and replicates, and their respective sampling time (season and year): colours represent stations, symbols represent sampling season and year. **B**: Shannon-Wiener diversity index for all stations and replicates: colours represent stations, larger circles reflect greater species diversity, and vice versa. Explained variation – PC axis 1: 20.74%, PC axis 2: 17.16%, explained variation (cumulative): 37.90%.

4.2 Shannon-Wiener diversity index

The role of impact and spring in reducing the SW diversity index becomes even more apparent in Figure 8: the greatest taxa diversity is centred around autumn and reference, whilst the lowest species diversity is located around spring and impact. This is especially true for three of the four red cases (lowest diversity) that are located in the first quadrant in close distance to spring and impact. However, the first axis explains roughly three times more of the variation than the second axis, indicating that season, which lies closer to the first axis, dictates species diversity to a greater extent than treatment.

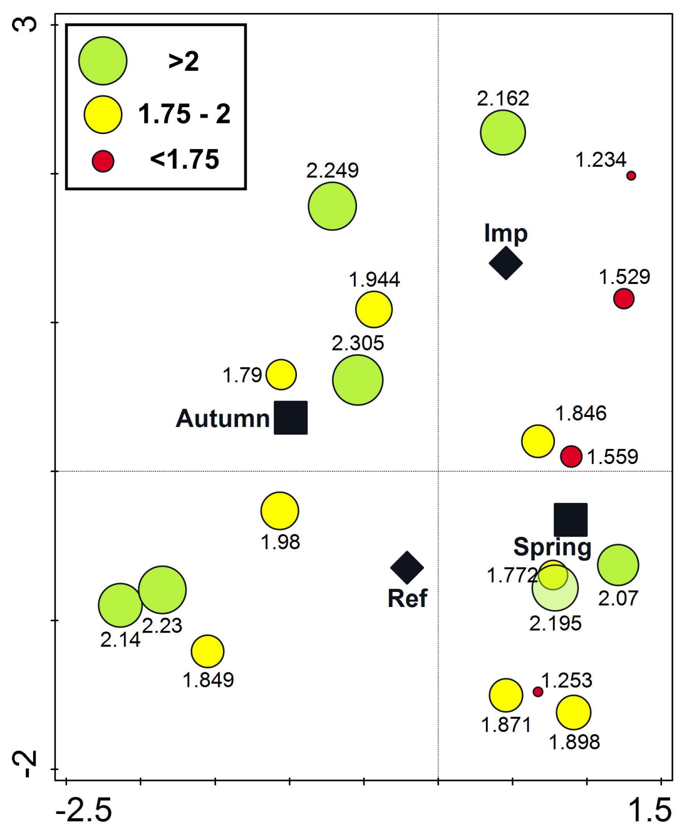

Figure 8 – Shannon-Wiener diversity index for macroinvertebrate data (RDA, 2.9 SD units long = linear), with season and treatment as explanatory variables. Higher number and larger circles = higher diversity. Based on station averages, replicates caused too many cases and overlaps. Explained variation - PC axis 1: 21.59%, PC axis 2: 6.72%, adjusted explained variation: 19.3%. First axis and all axes are equally significant (p = 0.002).

The model selection among candidate LME models fitted to explore effects from treatment and season on Shannon-Wiener index values gave highest support for an interaction model (i.e., Treatment*Season, Table 5). This model predicted reference stations to have significantly higher (i.e., 0.354±0.111 units) Shannon-Wiener index values during springtime, but not during autumn (Table 6, Figure 9). This result is also in accordance with the diversity pattern seen in Figure 7, where especially spring 2015 was characterised by a visibly lower diversity in the three impacted stations.

Table 5 – Ranked model selection table for candidate LME models fitted to Shannon-Wiener index values. K=number of fitted parameters, AICc = corrected Akaike's Information Criterion, ΔAICc= difference between AICc for a given model and the one with lowest AICc score, AICcWt=AICc weight (the relative support), LL=log likelihood value. The random effects model structure was random intercepts among replicate within station in all models.

Fixed effects model structure	K	AICc	ΔAICc	AICcWt	LL
Treatment*Season	6	16.92	0.00	0.9130	-1.57
Treatment+Season	5	22.97	6.05	0.0443	-5.86
Season	4	23.05	6.13	0.0426	-7.12
Intercept	3	35.14	18.22	0.0001	-14.33
Treatment	4	35.50	18.58	0.0001	-13.34

Table 6 – Parameter estimates and corresponding test statistics for the selected LME model in Table 5 fitted to predict Shannon-Wiener index values as function of treatment level and season. The random effect variance estimates: Between-stations: 0.0063, Between-replicates within-Station: 0.0626. Model fit: R^2_m=0.356, R^2_c=0.414. Treat=Treatment, Seas=Season, [R] = Reference sites, [A]=Autumn.

Parameter estimates			Analysis of Deviance (type III)			
Terms	Estimate	SE	Effect	F	Df.res	p-value
Intercept	1.393	0.093	Treat	1.963	24.773	0.1736
Treat[R]	0.354	0.111	Seas	19.544	46.227	<0.0001
Seas[A]	0.590	0.118	Treat*Seas	8.943	45.771	0.0045
Treat[R]*Seas[A]	-0.434	0.145				

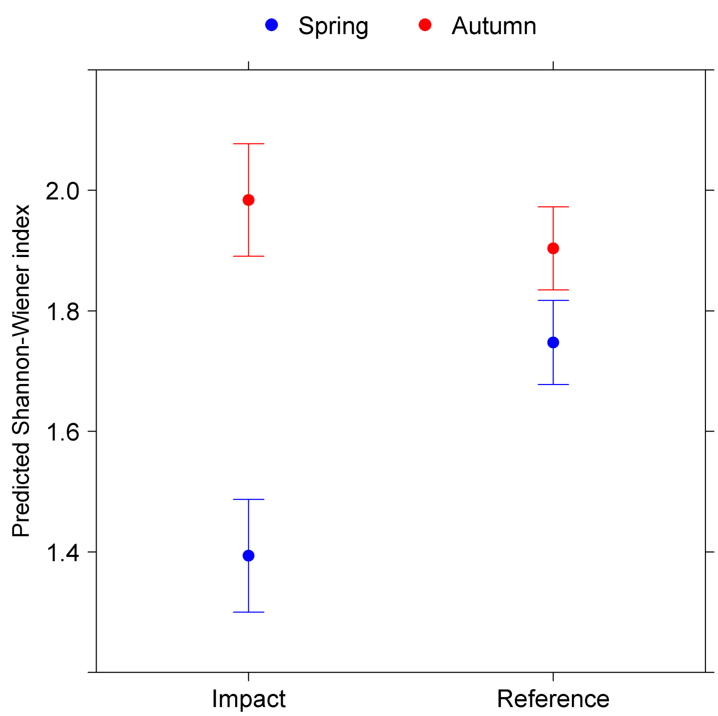

Figure 9 – Predicted benthic aquatic invertebrate Shannon-Wiener index scores from the Rv4 study area the as function of treatment and season. Predictions and corresponding 95% confidence intervals (error bars) were retrieved from the most supported LME model presented in Table 6.

4.3 ASPT index

In terms of ASPT index scores, some varying results were yielded (Figure 10). Autumn had almost consistently a higher score than spring across all stations. Vøien (reference) displayed the lowest interseasonal variation with all four scores qualifying for either a "good" or "very good" ecological quality class (QC). The score difference between spring and autumn was seemingly similar in Vigga upstream 2 (reference) and Nordtangen (impact), however, the former station was one ecological QC higher during both sampling rounds. There was little noticeable score difference when comparing the same seasons in 2013 and 2015.

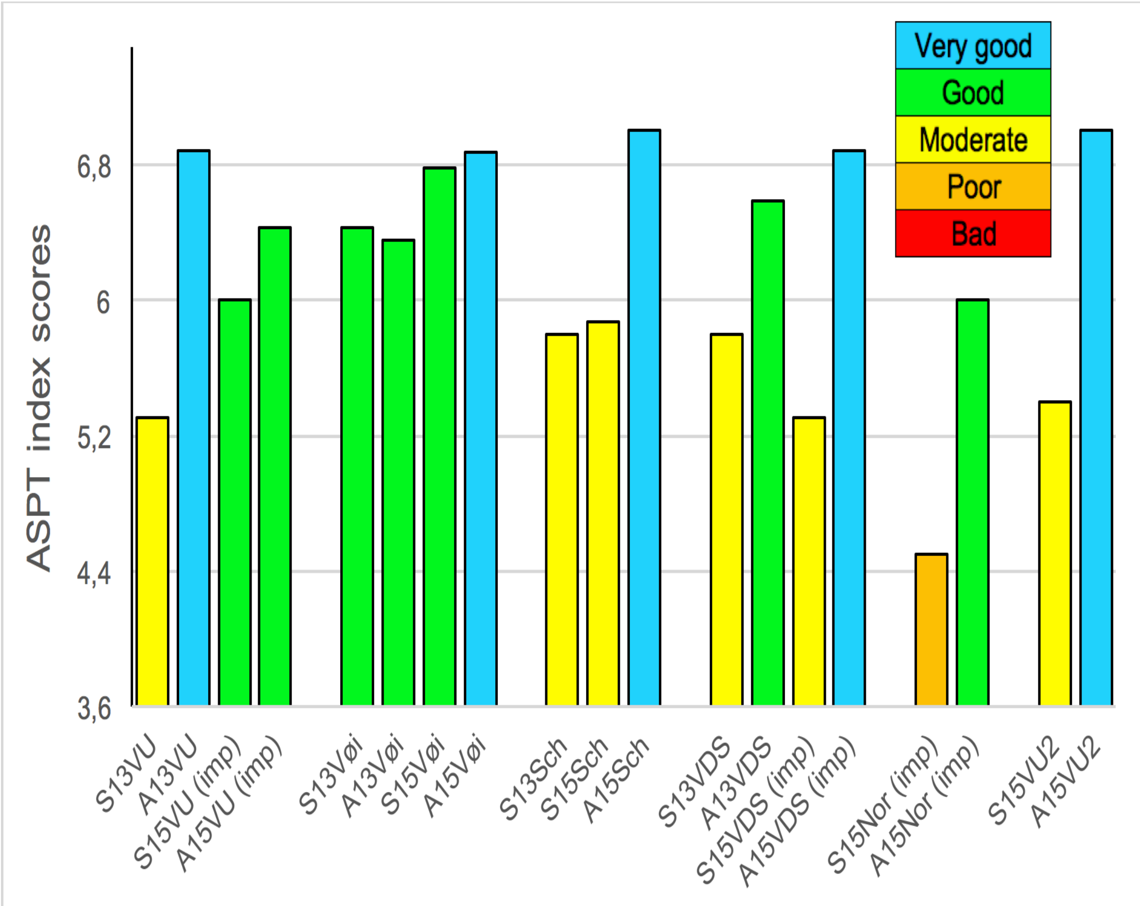

Figure 10 – ASPT index scores (applicable to Norwegian freshwater taxa) per station per field round. Ecological classes: < 4.4 = bad, 4.4 – 5.2 = poor, 5.2 – 6 = moderate, 6 – 6.8 = good, > 6.8 = very good. S = spring, A = autumn, 13 = 2013, 15 = 2015, VU = Vigga upstream, Vøi = Vøien, Sch = school, VDS = Vigga downstream, Nor = Nordtangen, VU2 = Vigga upstream 2. ASPT score table found in Miljødirektoratet (2013) and in Appendix 3.

The model selection among candidate LME models fitted to explore effects from treatment and season on ASPT values resulted in highest support for an additive model (i.e., Treatment+Season, Table 7). This model predicted reference stations to have significantly higher (0.898±0.198) ASPT scores than in both seasons, and autumn ASPT scores to be significantly higher (0.953±0.140) than spring scores (Table 8) for both treatment levels. This most supported model predicts that autumn comes out one ecological QC higher than spring for both treatment levels, and impact will be one ecological QC lower than reference regardless of season. ASPT score is predicted to be good during autumn in a reference area, and poor to bad during spring in an impacted area. Autumn in an impacted area is predicted to be the same (moderate) as spring in a reference area.

Table 7 – Ranked model selection table for candidate LME models fitted to ASPT values. K=number of fitted parameters, AICc = corrected Akaike's Information Criterion, ΔAICc= difference between AICc for a given model and the one with lowest AICc score, AICcWt=AICc weight (the relative support), LL=log likelihood value. The random effects model structure was random intercepts among replicate within station in all models.

Fixed effects model structure	K	AICc	ΔAICc	AICcWt	LL
Treatment+Season	5	99.20	0.00	0.5667	-43.97
Treatment*Season	6	99.74	0.54	0.4330	-42.98
Season	4	114.80	15.60	0.0002	-52.99
Treatment	4	130.19	30.99	0.0000	-60.68
Intercept	3	141.12	41.92	0.0000	-67.32

Table 8 – Parameter estimates and corresponding test statistics for the selected LME model in Table 7 fitted to predict ASPT values as function of treatment level and season. The random effect variance estimates: Between-stations: 0.1495, Between-replicates within-Station: 0.2593. Model fit: R^2_m=0.503, R^2_c=0.685. [R] = Reference sites, [A]=Autumn.

Parameter estimates			Analysis of Deviance (type III)			
Term	Estimate	SE	Effect	F	Df.res	p-value
Intercept	4.653	0.225	Treatment	18.071	46.78	0.0001
Treatment[R]	0.898	0.198	Season	46.334	46.464	<0.0001
Season[A]	0.953	0.140				

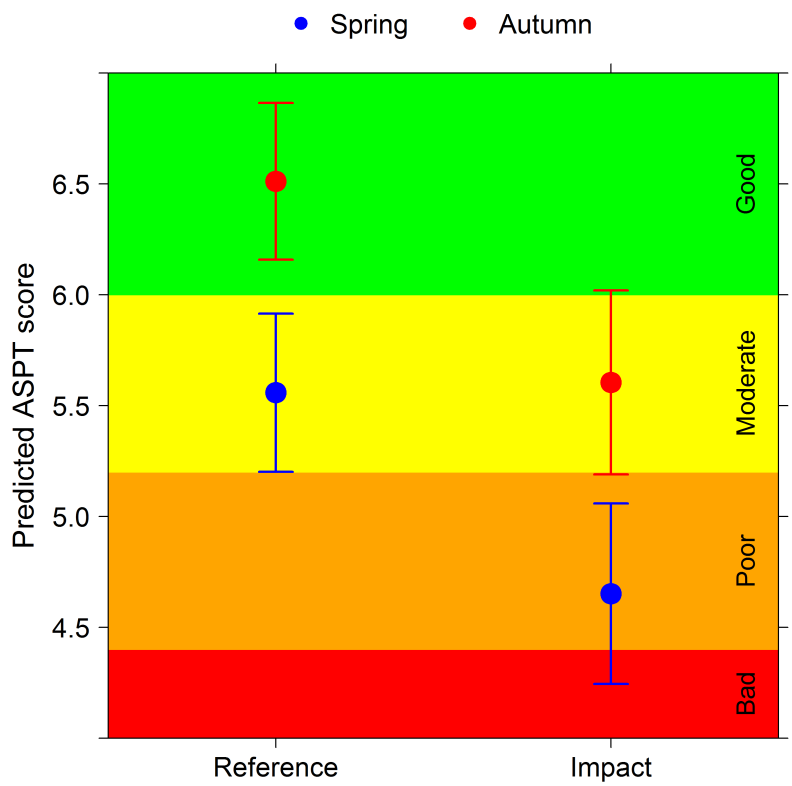

Figure 11 – Predicted ASPT scores from the RV4 study area as the function of treatment + season. Predictions and corresponding 95% confidence intervals (error bars) were retrieved from the most supported LME model presented in Table 8. Background colours represent WFD ecological quality classes (QCs) (see right vertical-axis).

4.4 Further ordination analyses

The effects season, year and treatment are more important than station in dictating the variation in the species data (Figure 12). First and second axis explain almost the same proportion of the variation and ~32% of all variation in the total data, and these three effects lie in very close proximity to these two axes. The six stations, however, are more widely distributed in the matrix and therefore appears to be an effect with little prediction power (will be omitted as effect in further species analyses). Spring and impact lie almost opposite autumn and reference, which is in accordance with previously observed patterns (for example, lower SW diversity index and ASPT scores for effects spring and impact).

The distribution of species also supports such a pattern: the majority of the taxa also branch towards autumn, reference and 2015, whilst much fewer taxa branch in the opposite direction towards spring and impact. When comparing against the ASPT score index, there is a relative overweight of tolerant taxa (i.e. chironomids (Bremnes 1991; Clements 1994)) on the positive side of the first axis whilst more sensitive taxa (i.e. *Leuctra spp*.) are located on the negative side.

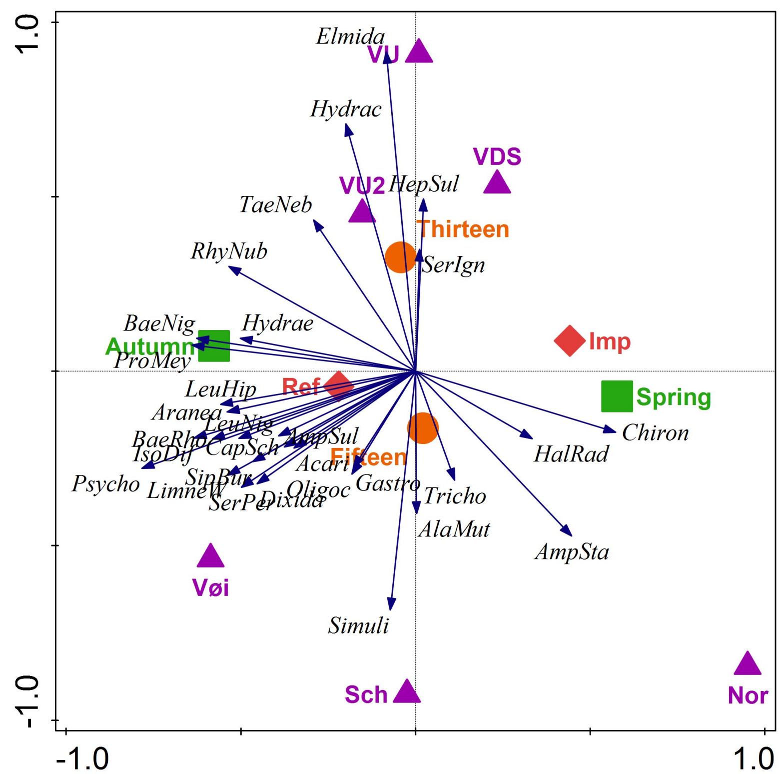

Figure 12 – RDA of species data with effects: stations (purple), seasons (green), year (orange) and treatment (red) as explanatory variables. Explained variation – PC axis 1: 16.82%, PC axis 2: 15.59%, adjusted explained variation across all axes: 40.9%. First axis and all axes are equally significant (p = 0.002).

All 14 mayfly metals that were measured were positively correlated along the positive side of the first axis, which explained as much as 84.22% of the variation (Figure 13, Table 9). The second axis contributed marginally with 7.29%, and its contribution to the total variation is therefore almost negligent. Closest to the first axis one finds aluminium, cadmium, nickel, cobalt and manganese, whilst the other important alum shale metals iron, thorium and uranium are less significant. Whilst the majority of cases are located at the negative side of the first axis, all spring 2015 cases are located on the positive side along with all metals; Nordtangen is more associated with uranium lead and thorium, whilst the other five stations are associated with heavy metals such as zinc, sulphur and copper. The three Vigga stations are in spring 2015 most associated with zinc and sulphur, with Vigga upstream and Vigga downstream being almost identical in their metal composition. Autumn 2013 is the season least affected by metal contamination. The case points from metal proxies PC1 and PC2 were extracted from this PCA for use as predictors in subsequent RDAs. With 84.22%, PC1 explained the vast majority of the variation, and was tightly correlated with the gradients of Al, Ni, Cd, Mn, Co and As.

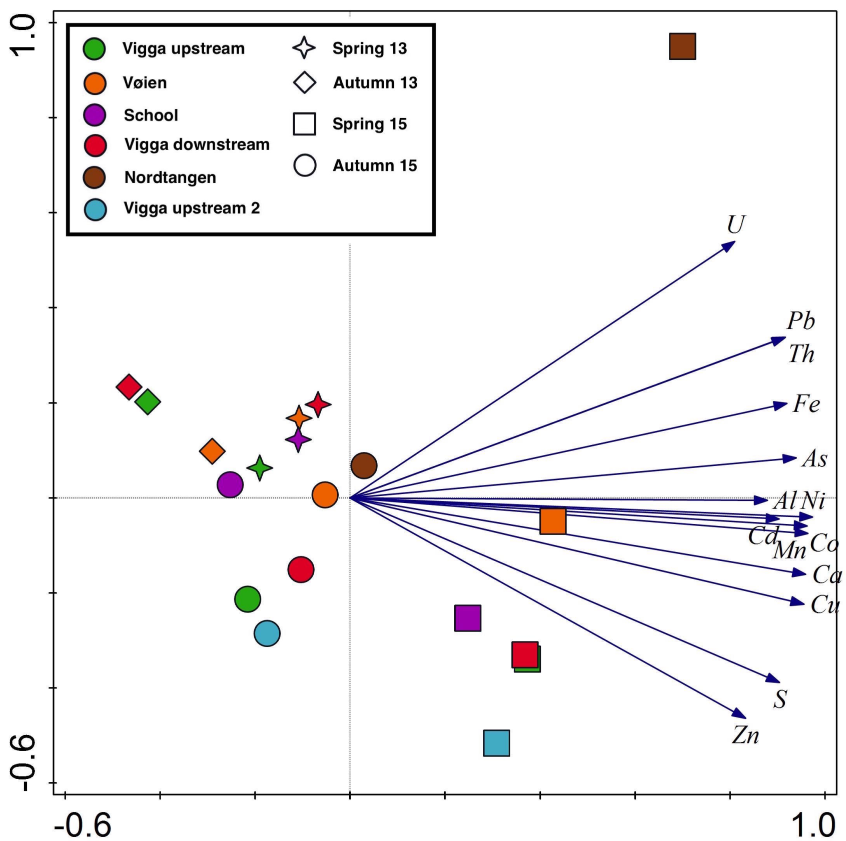

Figure 13 – PCA of metal concentrations in mayflies (analysis excludes station replicates, based on station averages so that stations could be more easily visualised) and the relevant placement of the different stations. Explained variation: PC axis 1: 84.22, PC axis 2: 7.29%. For the full names of the different metals, refer to List of Abbreviations on page 4.

Table 9 – Case points for all stations and metals (nuclides) for PC axis 1 and PC axis 2, as seen in Figure 13.

Sample ID	PC axis 1 (84.22%)	PC axis 2 (7.29%)	Nuclide	PC axis 1 (84.22%)	PC axis 2 (7.29%)
S13VU	-0.6171	0.205	Al	0.8781	-0.0049
S13Vøi	-0.3489	0.5451	S	0.9036	-0.3886
S13Sch	-0.354	0.3985	Ca	0.9589	-0.1611
S13VDS	-0.2196	0.6379	Mn	0.9639	-0.0748
A13VU	-1.3823	0.655	Fe	0.9195	0.1986
A13Vøi	-0.9421	0.319	Co	0.9621	-0.0593
A13VDS	-1.5089	0.7574	Ni	0.9737	-0.0407
S15VU	1.2121	-1.0998	Cu	0.9554	-0.2235
S15Vøi	1.3851	-0.1606	Zn	0.8322	-0.4634
S15Sch	0.8043	-0.8211	As	0.9386	0.0845
S15VDS	1.1938	-1.0715	Cd	0.9034	-0.0442
S15Nor	2.2709	3.0829	Pb	0.9152	0.3374
S15VU2	1.0007	-1.6735	Th	0.9163	0.3384
A15VU	-0.6998	-0.6907	U	0.8097	0.5396
A15Vøi	-0.1701	0.0211			
A15Sch	-0.8187	0.0887			
A15VDS	-0.3347	-0.489			
A15Nor	0.0955	0.2204			
A15VU2	-0.5663	-0.9247			

When the 14 metals were seen in relation to the effects in an RDA (Figure 14), a reoccurring pattern was seen; alongside the metal gradients one finds the effects spring, impact and 2015, whilst autumn, reference and 2013 are found on the opposite side. Here too the stations were used as an effect, however, they are all located very close to the second axis, which explains only 5.52% of the variation and twelve times less than the first axis. As with the species data, station as an effect is is not considered to have much prediction power over metal distribution.

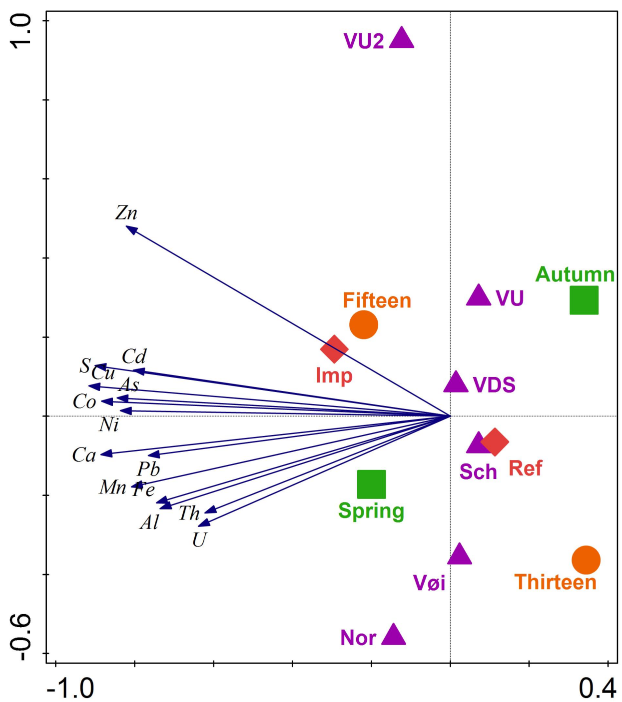

Figure 14 – RDA of metals (1.3 SD units long = linear) with season, year, stations and treatment as effects. Explained variation - PC axis 1: 67.53%, PC axis 2: 5.52%, adjusted explained variation: 72.9%.

Five metals commonly associated with alum shale bedrock – iron, aluminium, uranium, thorium and cadmium – were tested in an RDA (Figure 15) with season and treatment as correcting effects (using year as an effect in this test resulted in too many degrees of freedom). Here too one can see most of the taxa branch in the direction of autumn and reference, with the more tolerant taxa (i.e. families Chironomidae and Muscidae) and the five metal gradients going in the opposite direction towards spring and impact. The metals are all tightly clustered and positively correlated along the negative side of the second axis, which explains 14.25% of the variation and thereby 40% less than the first axis.

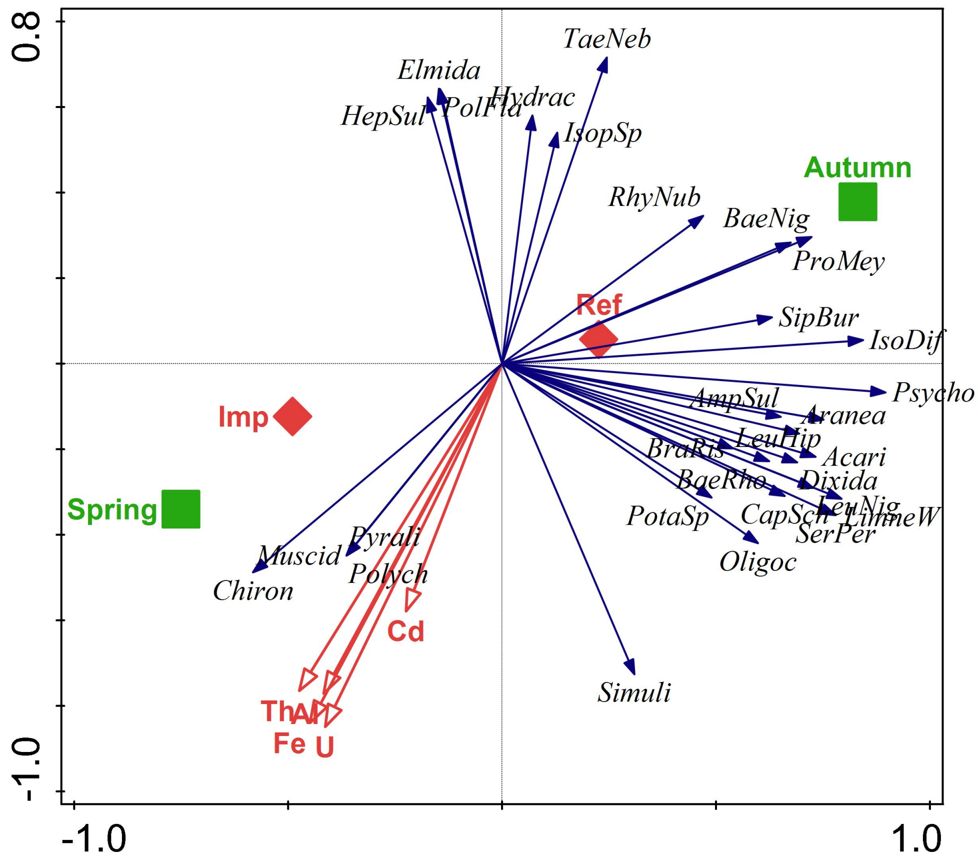

Figure 15 – RDA of species data (2.9 SD units long = linear) with five metals commonly associated with alum shale (U, Th, Al, Fe and Cd), season and treatment as predictors (no year, too many degrees of freedom). Explained variation - PC axis 1: 25.18%, PC axis 2: 14.25%, adjusted explained variation: 35%. First axis and all axes are equally significant (p = 0.002).

The importance of PC1 and the non-metal covariates temperature, conductivity and pH in determining species distribution was tested in an RDA with season, year and treatment as correcting effects (Figure 16A). Most of the taxa branch in the same direction as increasing pH, autumn and reference – all three located fairly close to the first axis, which explains 23.53% of the variation. Conductivity and temperature are, however, both located close to the second axis as well, which explains considerably less with 13.12%. Although the correlation seems weak, impact and spring are both characterised by a lowering in pH and conductivity, and an increase in temperature.

The increased metal concentrations are more associated with decreases in temperature and conductivity than it is with pH (Figure 16B). Also, they are all located closer to the second axis, which explains a mere 5.60%. The effects spring and impact seem to explain more of the variation than water quality does, and are located closer to the first axis which explains as much as 83.80%.

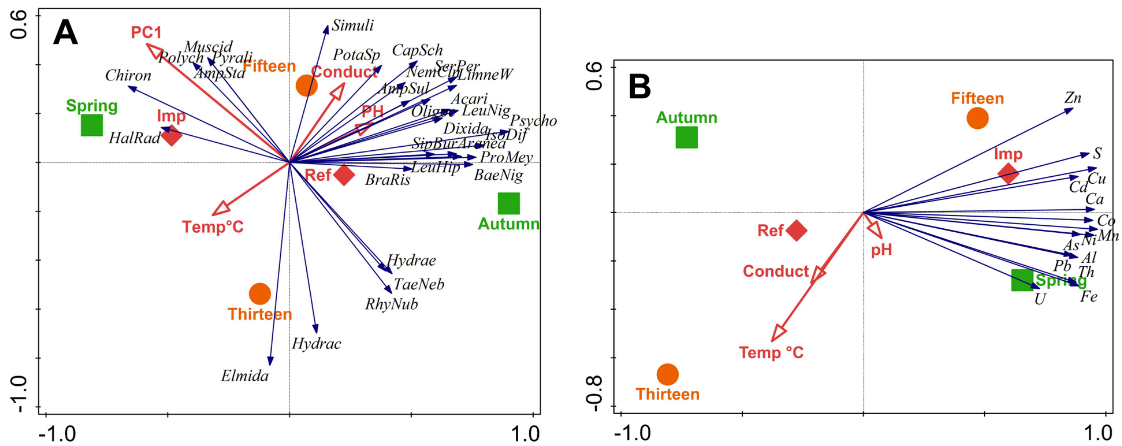

Figure 16 – A: RDA of species data (2.9 SD units long = linear) with PC1, conductivity, temperature, pH as covariates, and season, year and treatment as correcting effects (no PC2, too many degrees of freedom). Explained variation – PC axis 1: 23.85%, PC axis 2: 15.31%, adjusted explained variation: 37%. Significance: first axis (p = 0.004), all axes (p = 0.002). **B**: RDA of metal data (1.2 SD units long = linear) with season, year, treatment and water quality (conductivity, temperature, pH) as explanatory variables. Explained variation - PC axis 1: 83.80%, PC axis 2: 5.60%. First axis and all axes are equally significant (p = 0.002).

It is so far evident that the most important predictors in determining species composition are season, year, treatment and metals (represented as proxy values PC1 and PC2). In order to narrow these down to the three most important predictors, six different 3-group variation partitioning tests were executed (results are found in Table 10). The two RDA ordination diagrams in Figure 17 are the two tests that explain the most (A) and second most (B) of the variation in the data. The difference between the two tests is that test A includes the added effects of season and year in the same group, whereas test B only has season in the same group. By removing year as an effect and leaving only season, only

4.5% of all explained variation is lost (test 2). However, by removing season as an effect and leaving only year (test 3), an additional 0.4% of all variation is lost, leaving season as a marginally more important effect than year in this test. It has been safely established that PC1 explains considerably more of the variation than PC2, so by removing the latter one loses another 3.5% and is left with a total of 23.7% of all variation. These tests have now identified statistically that, under the aforementioned assumptions, the three most important drivers of species composition, in order of decreasing importance are: season (13.9 % of all variation), treatment (5.6%) and PC1 (4.3).

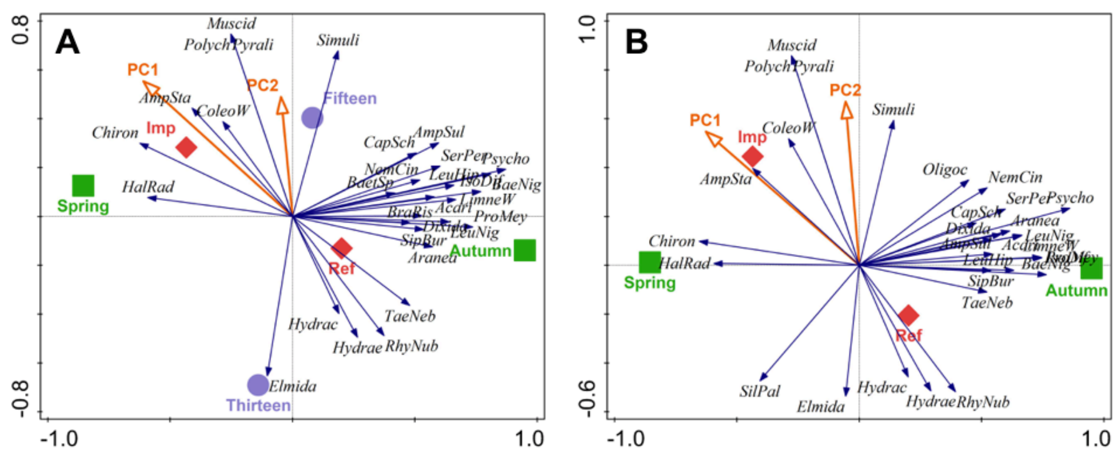

Figure 17 – The two most-encompassing predictor tests of the six 3-group variation partitioning tests (RDA, 2.9 SD units = linear). Out of six variation partitioning tests combining the most important variables, these are the two tests that explain the most variation (two top tests in Table 10). **A** – PC axis 1: 22.58%, PC axis 2: 11.04%, adjusted explained variation: 31.7%, all axes significant (p = 0.002). **B** – PC axis 1: 22.42%, PC axis 2: 9.39%, adjusted explained variation: 27.2%, all axes significant (p = 0.002).

Table 10 – Results for the six 3-group variation partitioning tests (2.9 SD units = linear), including *p*-values and % of all. Decreasing order from highest to lowest total explained variation.

Tests	***p*-value**	**% of Explained**	**% of All**
a (season + year)	0.002	61.5	19.5
b (PC1 + PC2)	0.008	25.3	8
c (treatment)	0.028	22.4	7.1
Total explained			**31.7**
a (season)	0.002	55.2	15
b (PC1 + PC2)	0.016	28.9	7.9
c (treatment)	0.022	22.8	6.2
Total explained			**27.2**
a (year)	0.002	54.5	14.6
b (PC1 + PC2)	0.002	76.7	20.6
c (treatment)	0.024	21.7	5.8
Total explained			**26.8**
a (season + year)	0.002	62.6	16.4
b (PC1)	0.016	9.9	2.6
c (treatment)	0.022	23.6	6.2
Total explained			**26.3**
a (season)	0.002	58.5	13.9
b (PC1)	0.052	18.3	4.3
c (treatment)	0.022	23.7	5.6
Total explained			**23.7**
a (year)	0.006	49.2	9.5
b (PC1)	0.002	67.7	13.1
c (treatment)	0.05	21	4.1
Total explained			**19.3**

All 85 taxa were divided into the main taxonomic group that they belonged to (Figure 18). These were the EPTs, as well as the class Oligochaeta, the order Coleoptera and the family Chironomidae. All taxa that did not belong under any of these groups were categorised as "other". The negative effect of increases in PC1 on ASPT scores is strikingly strong, as they branch almost completely in opposite directions along the first axis (34.66%). Increases in PC1 is associated with the effects impact, spring and 2015, whereas ASPT is associated with reference, autumn and 2013. An increase in PC1 has a positive effect on chironomids, but a negative effect on all other taxonomic groups. Somewhat surprisingly, year is the effect that explains most of the variation here.

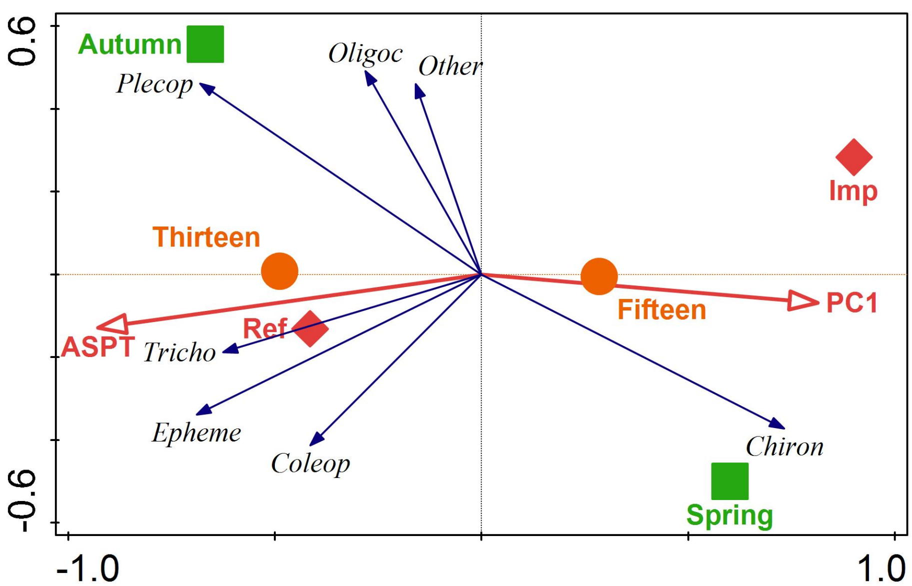

Figure 18 – RDA of main groups of macroinvertebrates (1.5 SD units long = linear) with PC1 and ASPT as covariates, and season, year and treatment as correcting effects. Explained variation – PC axis 1: 34.66%, PC axis 2: 15.49%, adjusted explained variation: 44.5%. First axis and all axes are equally significant (p = 0.002).

4.5 ASPT scores and mayfly metal concentrations

A significant negative correlation was found between nine mayfly metal concentrations (µg/kg) and ASPT scores (Figure 19). As metal concentrations increased in the tissue of the mayflies, the ASPT scores decreased significantly.

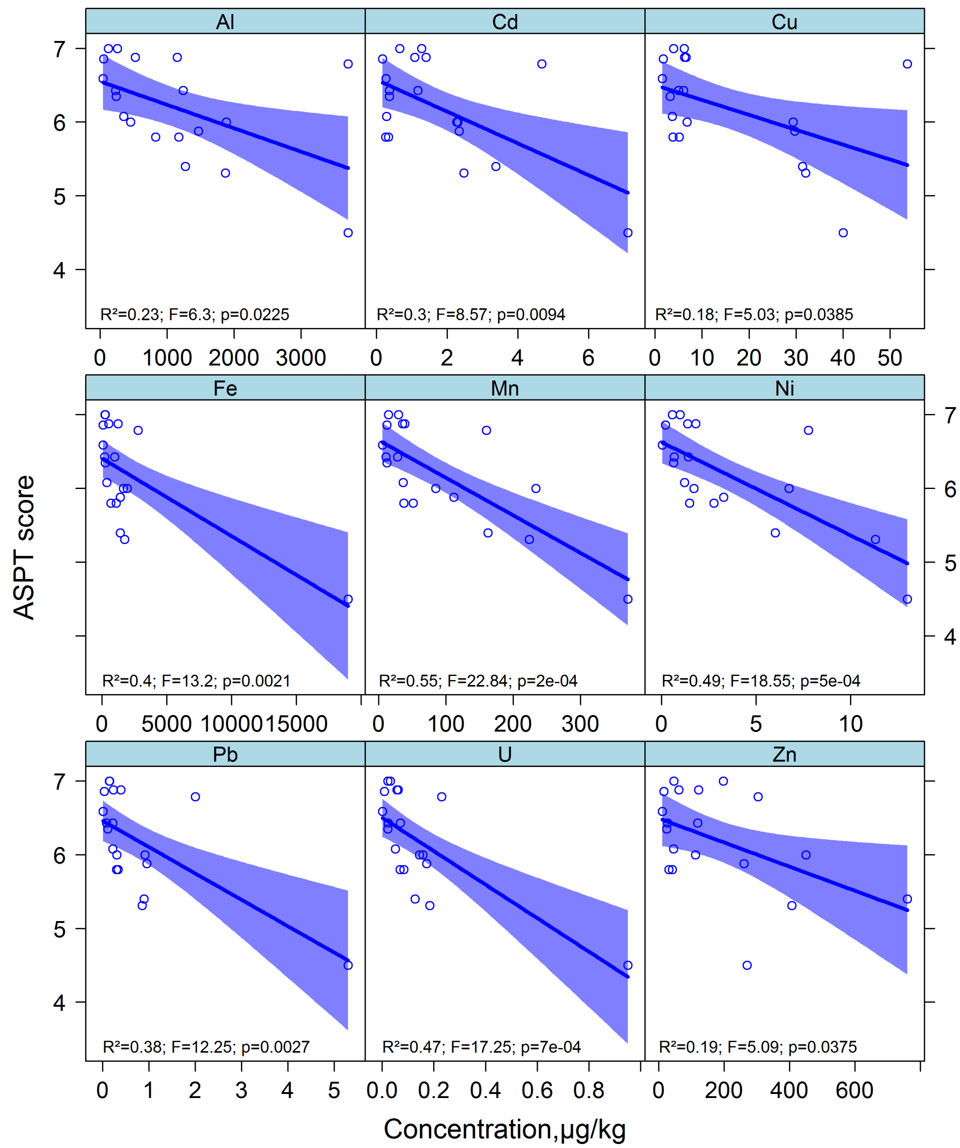

Figure 19 – ASPT score responses to increased concentrations of selected nuclides (metals and heavy metals) (µg/kg). Error bounds represent 95% confidence intervals for the fitted linear regression lines.

The same negative relationship between metals and ASPT scores as seen in Figure 19 was seen between metal proxy PC1 and ASPT scores. With p = 0.0013, the negative impact of increased PC1 on ASPT scores is highly statistically significant.

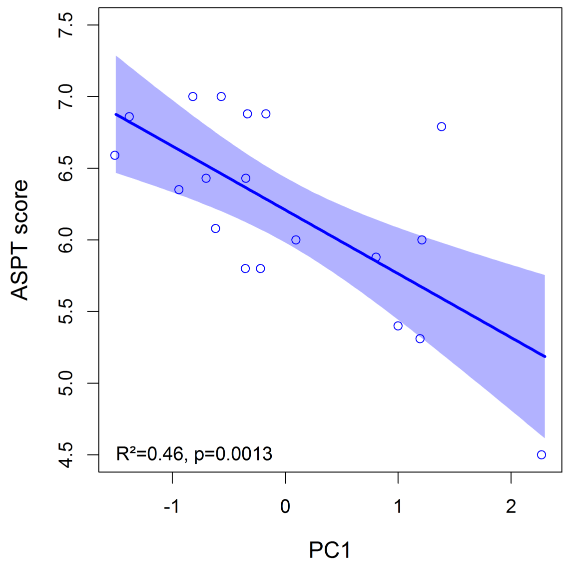

Figure 20 - ASPT score responses to increased concentrations of the metal proxy value PC1. Error bounds represent 95% confidence intervals for the fitted linear regression lines.

5 Discussion

5.1 Main findings

The main objective of this study was to assess if the Rv4 project has induced any significant ecological changes in the composition of benthic macroinvertebrate communities. Seasonal variability was found to be the single most important factor in determining the community structure of benthic macroinvertebrates. After correcting for season as an effect, the results clearly indicate that road construction associated with the Rv4 project has induced not only negative ecological changes in macroinvertebrate communities, but also elevations in mayfly metal concentrations in impacted areas.

The effects reference and autumn were generally associated with higher biodiversity, higher proportion of sensitive than tolerant species, higher ASPT scores, lower levels of metals, and a slightly increased pH. Impact and spring, however, were associated with the opposite. The vast majority of the taxa tended towards reference and autumn, many of which are considered to be sensitive species, such as the stoneflies *Taeniopteryx nebulosa*, *Leuctra nigra* and *Siphonoperla burmeisteri* (Miljødirektoratet 2013). The more tolerant taxa, such as the families Chironomidae and Muscidae, were positively correlated with the metal gradients and tended towards impact and spring. Mayfly metal concentrations were without doubt the highest (at all stations) during spring 2015, and concentrations had dropped considerably come autumn 2015. Metal concentrations in 2015 were higher than those of 2013 when comparing same seasons against each other (Figure 13).

Year as an effect was overall less accurate and also less important than season. However, year explained more of the variation than season did when one solely looked at EPTs and other major taxonomic groups instead of the 85 individual taxa (Figure 18). EPTs and ASPT scores favoured 2013 (reference) whilst PC1 and Chironomids favoured 2015 (both reference and impact). Covariates temperature, pH and conductivity did not explain much variation when compared to season, year and metals.

A significant negative correlation was found between ASPT scores and various metals, and ASPT scores were predicted to be one ecological QC lower in impacted areas than in reference areas. Nordtangen experienced extreme levels of iron deposition during spring 2015 (19,000 mg/kg), which had a measurable negative impact on biodiversity and sensitive species. The stonefly *Amphinemura standfussi* (97% of all EPTs) and Chironomids were both prevalent at this station. In the autumn of 2015 the iron levels at Nordtangen had dropped by 90%.

5.2 Rv4 project and its ecological impacts

The four main ecological features to look for when undertaking biological assessments of an area are 1) the number of individuals, 2) biodiversity, 3) the absence or presence of expected taxa, and 4) the ratio between tolerant and sensitive species (Persson et al. 2014). For example, finding a high proportion of tolerant species in an area, or alternatively observing a temporal community shift from sensitive to tolerant taxa, may be indicative of environmental disturbance (e.g. pollution).

Miljødirektoratet (2015a) defines environmental pollutants as chemicals that have no to low degradability, can bioaccumulate, and which may pose both long-term and short-term toxicity. A general response to pollution is a gradual change in macroinvertebrate assemblages as some tolerant species manage to adapt and proliferate whilst more sensitive species disappear, often causing a reduction in biodiversity but not necessarily abundance (Persson et al. 2014). However, the specific responses of macroinvertebrates and other organisms to external disturbance is highly dependent on the type of stressor (e.g. metal, organic, inorganic) as well as life-history strategies and stages (Johnson et al. 2006; Santos 2014). A typical response to metal pollution, which is a focal predictor in this study, is a proliferation in those species that are able to survive, usually because they are able to outcompete less tolerant competitors and predators (Bremnes 1991). A classic example of such highly tolerant species are chironomids, which are able to withstand organic pollution, very low pH values, and high concentrations of metals, such as Cu (Bremnes 1991). Oligochaetes are also quite tolerant to these impacts, however, they usually do not perform well in acidic environments (Bremnes 1991). In this study chironomids were abundant and always branched towards impact, spring and 2015, and along all metal gradients, whilst at the same time branching in the opposite direction of other, less tolerant taxa. This is a strong indication that the Rv4 project has had a negative impact.

5.2.1 Effect: Season and treatment

Based on the obtained results, it is evident that seasonal variation is the single most important factor in determining the community composition of benthic macroinvertebrates. Generally, the relative importance of seasons in dictating the occurrence and distribution of macroinvertebrate assemblages is small compared to spatial changes, such as channel width, maximum depth and velocity (Reece &

Richardson 1998). However, no such predictors were addressed here and the six stations were in close proximity to each other geographically.

Temperature is the environmental variable with the greatest impact on the life-cycle timing of benthic macroinvertebrate insects (Reece & Richardson 1998), making season essentially an indirect effect. Naturally, seasonal variation will have a major impact on community structure, especially in a high-latitude country like Norway with a wide range of climates and highly fluctuating annual and seasonal temperatures. This is also why it is recommended to collect macroinvertebrates twice yearly – once in the spring (after snowmelt) and again in the autumn (Persson et al. 2014). Hence, if seasons are not taken into account a lot of the explainable variation is lost, and looking at community structure statistically makes little sense unless natural seasonal variation is incorporated as a correction effect.

It is important to note that, the fact that spring-sampling actually took place in early summer and not during spring has likely influenced the results. Macroinvertebrates have different life-cycles, and for the EPT species, many may have already emerged as adults and left the station prior to sampling. In order to assess the extent of this, one would have to look at the respective life-cycles of each species, and even if the literature supports their presence at this station and emergence at this particular time of year, one would not know with certainty whether they were actually there or not. This unfortunate fact does cause some uncertainty in the spring data, and taxa richness and taxa diversity may therefore be an underrepresentation. It has also potentially contributed to the ASPT scores and SW diversity being lower during spring than during autumn.

When compared to spring and impact, the effects autumn and reference are associated with higher taxa richness, higher taxa diversity, higher proportion of sensitive than tolerant species, higher ASPT scores, lower levels of metals, and a slightly increased pH. There was no noteworthy difference in macroinvertebrate abundances between the two treatment levels. The vast majority of the 85 taxa tend towards autumn and reference, many of which are considered to be sensitive organisms, such as the species *Taeniopteryx nebulosa*, *Leuctra nigra* and *Siphonoperla burmeisteri* (Miljødirektoratet 2013). The more tolerant taxa, such as the families Chironomidae and Muscidae, are positively correlated with the metal gradients and tend towards spring and impact. Interestingly, on a general basis these results are somewhat in conflict with those of Jensen et al. (2014), who found that species richness in benthic macroinvertebrates actually increased in road-impacted streams adjacent to the stretch E134 Hokksund-Kongsberg-Notodden. However, the study also found that roads as a predictor had significantly affected the functional and taxonomic composition of macroinvertebrate communities, and that communities were generally different between impacted and control stations. This is also the case with the Rv4 project. Our studies have in common that the species *Amphinemura borealis*, *Amphinemura sulcicollis*, *Leuctra nigra* and *Baetis niger* were more abundant at reference stations. On the other hand, in their study *B. rhodani* was more common in impacted streams, which was the complete opposite of the results in this study.

ASPT scores were found to be a QC higher in the reference areas than in the impacted areas, and spring is expected to be a QC lower than autumn during both treatments (both $p < 0.05$). The data in this study also demonstrates higher ASPT scores in autumn than in spring. However, differences in spring and autumn ASPT can be expected, and possibly also attributed to episodes of lowered pH following spring snowmelt. It may also have been caused by the possible emergence of certain EPT species as was discussed earlier, but this is only a theory and far from being conclusive. The only relevant long-term pH measurements were from Vigga downstream and Vigga upstream, which both were impacted in 2015 – the former from treated tunnel water and the latter from general construction work. From October 1st, 2013 to November 1st, 2015, the pH at Vigga downstream and Vigga upstream ranged from 7.3 – 9.1 and 7.8 – 8.9, respectively (Leikanger et al. 2015a; Leikanger et al. 2015b). No long-term pH measurements exist for the remaining four stations. Based on the aforementioned pH ranges, it seems very unlikely that pH was the reason for spring having one QC lower than autumn, at least in the case of these two stations. The emergence theory therefore seems more likely.

Predicted SW diversity index, however, anticipates that there will be no statistically significant difference in species diversity between the two treatments in the autumn, but lower in impact than reference during spring. This may be attributable to reduced water quality (e.g. elevated metals and acidity) which is natural following snowmelt during spring, and because these levels will be further elevated in impacted areas. The emergence theory may also come into play here. These predictions are underpinned by the fact that measured SW diversity was higher near reference and autumn than it was during impact and spring (Figure 8).

Vøien acted as a reference station throughout the whole study, and it also had the highest number of individuals on average per replicate. This can perhaps be attributed to the heterogeneity and niche variety of the station, for example one large and deep pool, wide and narrow riffles, a small side-brook, and wide range of stream velocities. However, School, which was also a reference station throughout the whole study, had the third highest abundance. This station was quite homogenous when compared to Vøien, which potentially weakens that argument. Vigga downstream (directly impacted) and Vigga upstream (indirectly impacted) were the only two stations that acted as reference in 2013 and then impact in 2015, and the differences in abundance between the autumn samples in both of these stations were negligible. However, in the spring samples, the abundances in Vigga downstream was 60% higher in reference (2013) than in impact (2015). It is unclear what could have caused such a difference, whether it was due to random seasonal variability or perhaps increased metal concentrations.

5.2.2 Effect: Year

As 2013 acted as reference year and 2015 as impact and reference year, it is fair to assume that the average ecological state in 2015 should be equal to or worse than in 2013, after correcting for seasonal variation. This assumption proved to be partly true. While 2015 was clearly associated with increased concentrations of metals in mayflies and decreased ASPT scores, it was surprisingly more biodiverse than 2013 (Figure 12, Figure 16A, Figure 17A). This may just be a random response attributable to annual variation. However, if one solely looks at the distribution of EPTs, they branch towards 2013 (Figure 18). As most of the ASPT scores are based on these taxa, that might be the reason why 2013 scores higher in this regard. The importance of year as an effect seems to vary depending on what other predictors are included in the analysis, and much more so than with season and treatment.

Even though year clearly has an effect, the predictor-dependent inconsistency of this variable makes it less reliable. Also, year explained much less of the variation than season and treatment in all tests other than the EPT test (Figure 18); here 2013 and 2015 were perfectly aligned with PC axis 1 (32.66% of the variation), meaning that year was the most important effect in explaining the distribution of EPTs and the other major taxonomic groups. As chironomids was the only taxa that branched towards 2015 and all others branched towards 2013, one can at least conclude that a negative trend is seen in 2015 in regards to EPT and Coleoptera distribution.

However, it is important to note that future studies would benefit from including more years in their study design, thereby accounting for more seasonal and annual variability.

5.2.3 Covariate: Metals

Various metals, in particular mercury (Hg), lead and cadmium, are recognised environmental pollutants (Miljødirektoratet 2015a). Anthropogenic activities such as urbanisation, agriculture, urbanisation and mining often raise the levels of such metals beyond natural reference levels, and in excess they may cause widespread ecological management concerns in lotic environments (Iwasaki et al. 2009; Price 2013). As previously stated, benthic macroinvertebrate communities make for excellent bioindicators and their usefulness in assessing metal contamination through bioaccumulation has long been recognised (Clements 1994; Iwasaki et al. 2009). Despite large knowledge gaps, it is generally clear that short-term, and especially long-term, exposure to elevated levels of metals can have a host of negative and complex biological effects for benthic macroinvertebrate taxa (Chiba et al. 2011). Most metals and radionuclides can, some even at relatively low doses, interfere with important physiological functions of fish, such as the olfactory system and the exchange of gases and ions (Hjulstad 2015; Price 2013). Although much more is known about the direct effects of metals rather than sub-lethal effects, such as predator avoidance, migration and foraging (Price 2013), it is nevertheless recognised that elevated levels of dissolved metals can severely reduce the fitness and survival of an organism. To underpin this, Durrant et al. (2011), for example, found that brown trout avoided the middle section of a river in a mining-region, which happened to also be the part of the river most contaminated by metals. Most studies have focused on the biological effects of copper, cadmium, lead and nickel – metals which also commonly originate from processes such as road traffic and combustion reactions (Næss 2013).

Bioaccumulation – the accumulation of a substance or chemical in a living organism – is well studied and documented biological process in aquatic organisms (Goodyear & McNeill 1999). However, the respective responses of macroinvertebrates and general biota to changes in specific contaminants, such as metals, have generally been neglected in recent research, and future conservation efforts must therefore rely on a very inadequate knowledge base (Strayer 2006; Wheeler et al. 2005). Considering the fact that human activities are increasingly threatening freshwater resources globally (Chiba et al. 2011) and that freshwater taxa are going extinct at an unprecedentedly fast rate, this is a field that urgently should receive some much-needed attention (Ricciardi & Rasmussen 1999; Strayer 2006). However, this seems to be an up-and-coming field of research at the time of writing.

As previously mentioned, this study has demonstrated that mayfly metal concentrations are elevated in areas impacted by Rv4 construction activities when compared to reference areas, which is in accordance with the findings by various other studies. For example, by measuring runoff in brooks along the new E6 Oslo – Trondheim highway, Næss (2013) found considerably higher levels of heavy metals in watercourses within the construction area than in those outside that were not impacted.

Hindar (2011) assessed the impacts of acidic runoff stemming from three different deposits related to the new highway E18 Grimstad-Kristiansand, which was completed in 2009 after a three-year long construction phase. Sections of the road was built in an area with sulphide-rich bedrock, and oxidation of excavated material caused the formation of sulphuric acid. This lowered the pH of downstream Lake Lomtjenn significantly compared to background levels, which in turn increased aluminium and trace metal concentrations by a factor of 25-50 and 25-400, respectively. This runoff caused conditions that were too toxic for resident fish species to survive in, and the lake did not recover even after countermeasures to increase pH were taken in 2009.

Although knowledge about the effects of metals on macroinvertebrate communities is currently limited, a study by Kashian et al. (2007) is very interesting to point out: In microcosm experiments with benthic macroinvertebrates it was found that a gradual increase in community-level tolerance to metal pollution resulted in a reduced tolerance to genetically deleterious UV-B radiation (especially in mayflies). This is an issue considering ozone depletion in the Northern Hemisphere has caused a significant increase in UV-B radiation following the advent and worldwide application of ozone-damaging compounds such as chlorofluorocarbons (Kerr & McElroy 1993). In the control microcosms, however, macroinvertebrates did not perform well when exposed to metals, but their tolerance to UV-B did not change. Hence, exposure to a specific contaminant may prompt an organism to spend extra energy for maintenance and adaptation, thereby making itself more susceptible to the detriment of additional stressors, such as acidification (Kashian et al. 2007). Unfortunately, most such studies have focused only on single disturbances rather than the effects of multiple disturbances, and experimentally investigating effects over multiple generations is highly complicated to facilitate (Kashian et al. 2007).

Long-term exposure may cause persistent structural changes to macroinvertebrate assemblages, potentially encouraging metal-tolerant communities to form (Kashian et al. 2007). For example, studies of various regions where mining activities have occurred in the past show that high concentrations may chronically persist in the surrounding aquatic environment for decades after (Durrant et al. 2011). Bremnes (1991) found that lakes adjacent to Vigsnes copper factory at Karmøy, Norway, which was abandoned in the early 1970s, were strongly contaminated by copper and zinc, both in the water and in sediments, even twenty years later. The conditions in the lakes were generally unfavourable for fish and other biota, and only the hardiest macroinvertebrates were present at varying densities. These were mostly chironomids, however, their larvae were smaller than usual, which is likely attributable to the combined effects of multiple stressors, such as heavy metals and low pH.

Studies have shown that it is possible to generalise a positive relationship between environmental and animal tissue metal concentrations (Goodyear & McNeill 1999). For example, in a review study by Goodyear and McNeill (1999), concentrations of Zn in water and in insect larvae were found to be significantly positively correlated, and sediment concentrations of zinc, copper and lead were directly proportional to organismal concentrations. This link was especially strong for cadmium concentrations. Qu et al. (2010) studied heavy metals effects on benthic macroinvertebrate communities in high mountain streams in China, and found that increases in heavy metal concentrations altered species composition and caused a decline in total abundance and species richness (especially sensitive genera of Trichoptera and Plecoptera). Interestingly, the same study also found that certain genera of Trichoptera, for example *Polycentropus*, were tolerant and increased with increasing cadmium concentrations. However, while *Polycentropus* was present in the Rv4 study, only a very few individuals were recorded and they only occurred in the autumn. Similar effects were reported by Iwasaki et al. (2009), who's research demonstrated a significant negative response of benthic macroinvertebrate diversity and abundances to mining-induced heavy metal pollution (zinc, copper, cadmium and lead) in northern Japan. This in turn had a negative impact on the recruitment and conservation of stream-living fish, who tend to be highly dependent on macroinvertebrates as a food source (Iwasaki et al. 2009). This may also have ecological and economic consequences for Norwegian trout and salmon-bearing watercourses.

In the case of this study, the five metals Al, Cd, Fe, Th and U were positively correlated to the degree that they were almost indistinguishable in their ordination cluster, and they all branched in the direction of impact (Figure 15). The chironomids were always found in close proximity to these metals as well as PC1. This pattern is in accordance with the findings of Iwasaki et al. (2009), although Cd was the only metal the studies had in common. The concentrations of cadmium, and especially the proportion of free Cd^{2+}-ions, is very important to measure in regards to pollution as it is one of the most toxic metals known at high concentrations (Price 2013; Xue & Sigg 1998).

Environmental pollutants end up in waterways and waterbodies mainly through three mechanisms – precipitation/atmospheric deposition, runoff and from local emissions/non-point sources (Chiba et al. 2011; Miljødirektoratet 2015b). Due to atmospheric long-distance transport, many environmental pollutants, including heavy metals, actually originate far from the water source in which they are measured, and, for example, the national nutritional mercury warning that has been issued is mainly due to such long-distance transport (Miljødirektoratet 2015b). Although the results show that metals are associated with impact, they are also associated with spring. This time of year discharge volumes are high due to snowmelt in the catchment, and metals that have been deposited over the course of the winter are transported downstream with the runoff. This has likely contributed to the increased concentrations of metals during spring.

There are currently no national benchmark or reference values for freshwater metal concentrations in macroinvertebrates, but they do exist for water and sediments. However, it is clear that any levels of metals beyond what is normal for a particular aquatic system (taking into account seasonal and annual variability) is not beneficial, and such watercourses should be managed accordingly. Such seems to be the case for the Rv4 project.

In the 3-group variation partitioning test executed to identify the most important predictor (Table 10), season is shown to explain 13.9% of all variation in the macroinvertebrate data (p = 0.002) when compared to PC1 (4.3%, p = 0.052) and treatment (5.6%, p = 0.022) (Test 5). In total the three predictors explain 23.7% of all variation. Although the majority of this variation can be attributed to seasonal variability, the effects of treatment and PC1 were significant and almost significant, respectively. If the effects of PC2 is taken into account as well (Test 2), the two metal gradients combined become significant (7.9% of all variation, p = 0.016). In other words, treatment and the combined effects of metals PC1 and PC2 have had a significant impact on the macroinvertebrate community in this study.

5.2.4 Nordtangen (Nor)

Although not being directly impacted by the Rv4 project (e.g. no direct discharge of treated water), Nordtangen was arguably the most affected station. The tiny and narrow brook runs through a densely vegetated forest and is characterised by small discharge volumes, short riffles and small pools (Appendix 4). During spring the station was highly impacted by iron depositions (19,000 mg/kg), which was very visible as oxidised iron (rust) (Figure 21). This impact was much less pronounced in the autumn, where the concentrations had dropped to a tenth of the spring level. The ASPT score was during spring the lowest of all samples, but during fall it increased by two QCs. Based on the ASPT LME model, scores are predicted to be one QC higher during autumn than during spring. The fact that it was as much as two QCs lower in the spring can probably be linked to the high iron deposition.

Figure 21 – Iron deposition (rust colour) at Nordtangen during spring 2015.

5.2.5 Vigga downstream (VDS)

Vigga downstream was exceptionally turbid on the day of the field work in autumn 2015. The stream was coloured grey and there was zero visibility (Figure 22, Appendix 4). It was later revealed that this was caused by a direct release of untreated particle-rich water from a construction pit next to the tunnel earlier in the day. Although it is known that the pit was dug in the Elnes Formation, the exact impact it had on the results is unknown. Considering the time span and that the metal concentrations were measured in mayflies and not in the water, this event should have little to no effect on metal levels. However, a team of NMBU researchers had to abandon their electrofishing plans due to this increased turbidity. One small crayfish ended up in the kick net this day.

Figure 22 – Vigga downstream after the release of untreated pit water.

5.3 Vigga – the past and present

The area that includes the municipalities of Gran and Lunner is largely cultivated land which is very rich in calcium, and the lakes in the area also have very high calcium levels (in this case Chara lakes, in which the algae muskgrass/*Chara* is prevalent) (Rustadbakken et al. 2011). A great proportion of this land lies within the Vigga watershed and thereby acts as a source of sludge and organic loading to Vigga (Rustadbakken et al. 2011). In 1991, a third of the Vigga watershed was cultivated land (Rustadbakken et al. 2011).

In late fall of 2010, three years prior to commencement of the Rv4 project, Rustadbakken et al. (2011) undertook an ecological assessment of Vigga, with emphasis on communities of macroinvertebrates, fish (trout and minnow) and crayfish. Although they assessed the conditions for macroinvertebrate production to be healthy and diverse, the densities of fish and crayfish were well below what can be expected in a river like Vigga.

They were, however, unable to identify specific bottlenecks for trout and crayfish production, but they concluded that low recruitment was likely due to morphological changes in the river and deterioration of beneficial habitats as a result of previous flood control measures. The ecological state ranged from being bad to very bad, however, different indices were used and these results are therefore difficult to compare with. The ratio between EPTs was fairly similar to the findings in this study, with mayflies being the most abundant order, which is normal. As this was purely an ecological assessment, no emphasis was put on water quality or metal concentrations in macroinvertebrates.

Of the three Vigga stations, Vigga downstream had the lowest macroinvertebrate abundance, which can be expected in areas where the number of anthropogenic sources increase towards the river mouth (Rustadbakken et al. 2011). This pattern was also noticeable in metal concentrations, where concentrations in Vigga increased downstream. The macroinvertebrate communities at the three Vigga stations were much more similar to each other than they were to those of the other three stations, regardless of season and treatment. This could be expected as all three stations occur in the same watercourse.

As mentioned earlier, the pH ranged from 7.3 – 9.1 at Vigga downstream, and considering that fish are normally unaffected by pH 5 – 9 (NFF 2009), it is unlikely that the pH had any negative effect on fish in Vigga.

However, when interpreting the results in this study, it is important to keep in mind that Vigga has a long history of being impacted by surrounding anthropogenic activities.

5.4 ASPT as an indicator of metal contamination

The ASPT index is commonly employed as an indicator of eutrophication and organic enrichment in a water body (Clements 1994; Zeybek et al. 2014), and also often in the context of general water quality. Its applicability in watercourses affected by metal contamination is, on the other hand, more unsettled. For example, one can generalise that some of the common EPTs may display a sensitivity towards organic pollution, whilst tolerant species, such as Chironomids, will proliferate in nutrient enriched areas

(Clements 1994). However, if metal contamination is the culprit, such a generalisation may no longer be valid and responses have been found to vary on a species to species level, regardless of order. Attempts to find studies that have looked at correlations between metal levels and ASPT scores have so far been unsuccessful.

However, this study has demonstrated a clear significant negative response of ASPT scores to increased concentrations of metals PC1 (Figure 18, Figure 20) and Al, Cd, Cu, Fe, Mn, Ni, Pb, U and Zn (Figure 19). This is exemplified by Nordtangen during spring 2015, which was arguably the most contaminated station (i.e. orange substrate from oxidised iron deposition, 19,000 mg/kg). It also had the lowest ASPT score and the lowest taxa diversity. The EPT ratio (%) at this station was 2/97/1, and the overwhelmingly over-represented Plecoptera proportion was made up by 60 *Amphinemura standfussi* and only one *Nemoura cinerea*.

It should be noted, though, that while these results do demonstrate a correlation between metals and ASPT scores, they do not demonstrate a causation. A large proportion of the Vigga watershed is, after all, cultivated land on which synthetic fertilisers get applied. Unfortunately, no measurements of nitrogen and phosphorus compounds were taken and therefore it is difficult to conclude whether or not the decline in ASPT score was also induced by organic enrichment (there were no obvious visible signs of eutrophication during field work).

Nevertheless, these results do validate the use of mayfly metal concentrations to predict ASPT scores in stream environments.

5.5 Water quality shortcomings

As previously stated, there are certain interpretational shortcomings associated with using water quality (e.g. pH, conductivity, temperature) and water metal concentrations as covariates in this study. This is especially true for streams due to their unidirectional flow; when a contamination event is stopped or halted, it is just a matter of time (i.e. seconds, minutes, maybe hours) before all chemical traces have drifted further downstream and away from the contamination epicentre/release point. These are by nature immediate measures, and this fact renders water quality a potentially coincidental and inaccurate measure, especially if not taken at the exact same time as the kick-sampling. However, certain environmental variables can only be sampled from water (in this case pH, conductivity and temperature), but the timing and precision of water quality measurements is key.

Another issue is that there is no way of backtracking the values of the three water quality variables or interpreting how they behaved prior to reaching the stations in which measurements were taken. For example, a preferred pH of wastewater reaching the recipient will be about 8 (Næss 2013). The *in situ* pH measurements at our stations (not immediate, but measured the same day as kick-sampling) ranged from 7.85 – 8.7, which is not nearly acidic enough to cause problems through mobilisation of metals from the sediments. However, this was the pH measured in the streams and therefore does not give any indication of the runoff pH nor the pH that occurred right when water and oxygen first made contact with acid-forming bedrock materials (e.g. sulphur containing minerals). This runoff may have been highly acidic prior to reaching downstream watercourses, and, if so, could potentially be the reason why metals were elevated in impact areas. After all, elevated levels of mayfly metal concentrations were associated with impact.

However, by assessing the immediate benthic macroinvertebrate community, larvae in particular, one might get a much more accurate picture both temporally and spatially. Several insect larvae and other macroinvertebrate taxa are fairly sedentary and unable to move far from their origins, which makes them good representatives of local conditions (Goodyear & McNeill 1999). They are also often associated with sediments and situated near the very bottom of the aquatic food chain, which are both important points of origin for metals to enter the food chain (Goodyear & McNeill 1999; Santoro et al. 2009). However, these organisms are clearly not useful for covariates such as pH, conductivity or temperature, for which water is the only thing it makes sense to test.

5.6 Further study limitations & considerations for future studies

It is important to keep in mind that abundances in this study are estimates at best. Kick-sampling is a semi-quantitative method, meaning the number of macroinvertebrate individuals in each replicate is more an abundance estimate (with associated potential biases and sampling errors) than it is an exact value. This makes the count data a less accurate representation of actual biotic conditions, however, retrieving exact values is practically an impossibility in natural stream environments.

The samples from 2013 experienced some tagging issues as well as loss of replicates. For these reasons there ended up being exactly twice as many samples from 2015, which unfortunately causes the statistical power of the tests to become somewhat reduced.

In order to correct for annual variability, comparing changes in benthic macroinvertebrate communities over the duration of several years can be beneficial, assuming sampling is standardised, e.g. occurs at the same time each year (Beatty et al. 2006; Hayslip 2007). More accurate results would have been yielded if the study started earlier and lasted for a few years after Rv4 project ended (which would have included more seasons), as opposed to only having one reference year and one impact year. Sampling one season over four years, for example, instead of two seasons over two years, will remove interseasonal variation from the equation, and the individual effects of the two treatment levels can potentially become more apparent. However, doing so will also extend the duration of the project, which may not be beneficial in respect to planning and budgeting.

In the view of the author, the best and most ideal approach to quantify construction impacts would be to sample one season (or several) over as many years as possible, and then compare the communities and covariates of each season with those of the same season each year (i.e. spring 2013 vs spring 2014 vs spring 2015). This could be a better approach for isolating the effects of construction-related impacts. If the objective is to get a picture of how macroinvertebrate assemblages change with seasons, then the sampling methodology undertaken in this study would have been the best way to go. However, since the main objective in this study was to establish if the Rv4 project has had any ecological impacts on benthic macroinvertebrates, comparing the same seasons with itself over the duration of multiple years would arguably have yielded better and more interpretable results.

5.7 Conclusion

With reference to the stated objective, this study has demonstrated that the construction activities pertinent to the Rv4 project has caused negative ecological changes to benthic macroinvertebrate communities. This is in accordance with the findings of various other studies focusing on issues such as freshwater metal contamination, impacts of road construction and use, and biotic responses to alum shale excavation and mining.

In general, although to varying degrees, when compared to reference areas, impacted areas were associated with lower taxa richness, lower taxa diversity, much higher proportion of tolerant than sensitive species, lower ASPT index scores, elevated levels of mayfly metals, and a slightly decreased pH. There was no noteworthy difference in abundances across treatment levels. Based on the available literature, these effects can often be expected.

Impacts can also be attributed to seasonal and annual variability, with spring and autumn sharing macroinvertebrate community similarities with impact and reference, respectively. The largest impact occurred during spring 2015, which affected all stations regardless of their treatment level. The impacts were the most striking at Nordtangen as iron concentrations were tenfold higher than they were during the fall. Surprisingly, larvae of the stonefly *Amphinemura standfussi* was relatively abundant here, with mayflies and caddisflies being virtually non-existent. Impacts were likely more severe during spring than autumn due to increased runoff from rain and snowmelt, which causes increases in acidity and dissolved metals.

Mayfly metal concentrations were also elevated in spring and in impacted areas, and the five alum shale metals Al, Cd, Fe, Th and U, as well as metal proxy PC1, were positively correlated and clustered tightly in this direction. Impacted areas and spring were also highly associated with tolerant taxa, especially chironomids, whilst reference areas and autumn were associated with sensitive species, such as *Taeniopteryx nebulosa*, *Leuctra nigra* and *Siphonoperla burmeisteri*. EPT taxa strongly favoured 2013 over 2015, were positively correlated with increasing ASPT scores, and were affected negatively by elevated metal concentrations. The finding that ASPT scores were lower in spring may be partly due to the emergence theory.

A bit unexpectedly, ASPT index scores were found to be significantly negatively correlated with mayfly metal concentrations (all tested metals and PC1). This validates the ASPT index as a good indicator of metal contamination.

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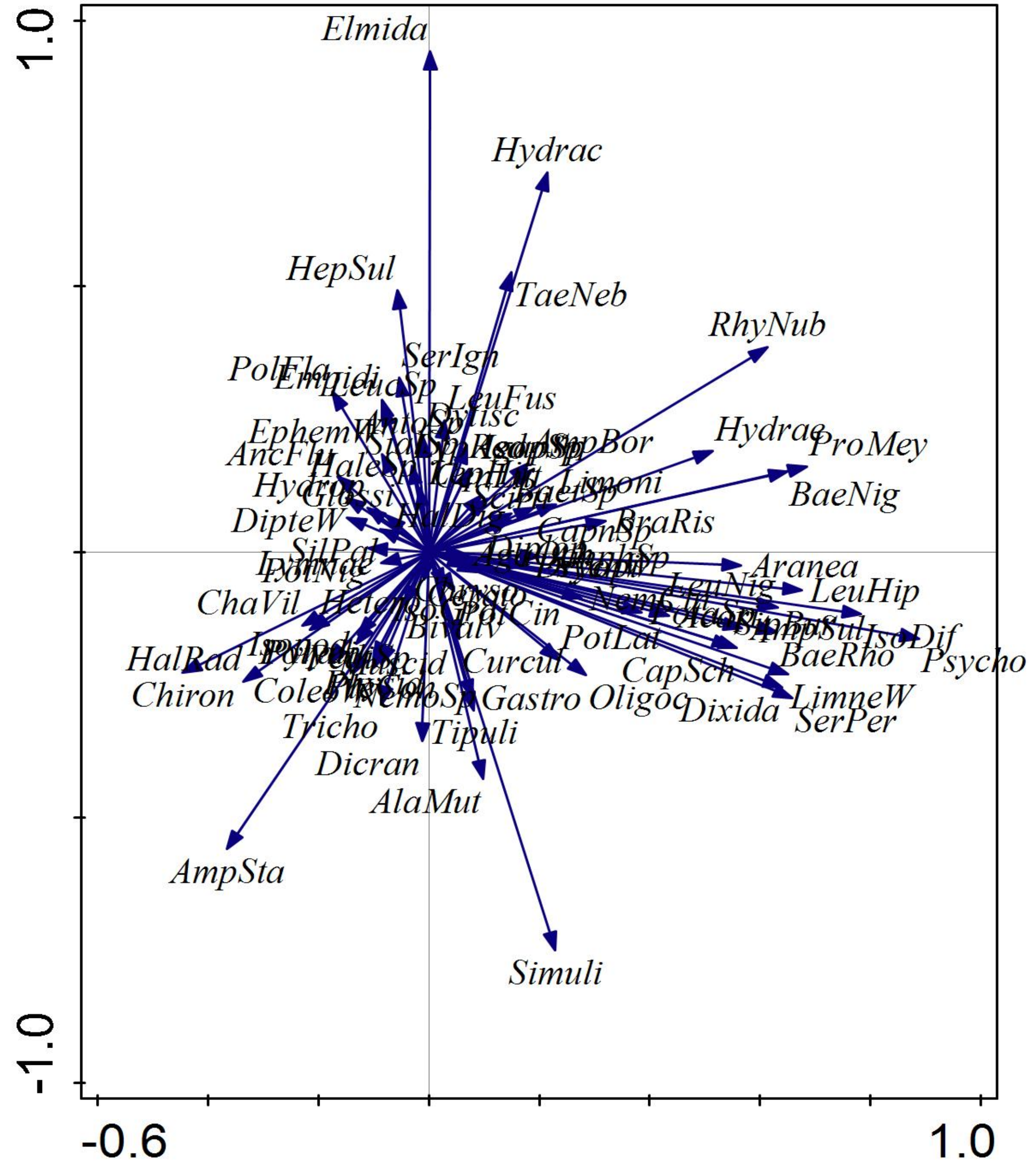
7 Appendix

7.1 Appendix 1 – Original and modified names and codes for taxa

Original code	Original name	Modified code	Modified name
Acari	Acari	Acari	Acari
Aranea	Aranea	Aranea	Aranea
AlaMut	Baetidae, Alainites muticus	AlaMut	Baetidae, Alainites muticus
BaeNig	Baetidae, Baetis niger	BaeNig	Baetidae, Baetis niger
BaeRho	Baetidae, Baetis rhodani	BaeRho	Baetidae, Baetis rhodani
BaetSp	Baetidae, Baetis sp.	BaetSp	Baetidae, Baetis sp.
CenLut	Baetidae, Centroptilum luteolum	CenLut	Baetidae, Centroptilum luteolum
Bivalv	Bivalva	Bivalv	Bivalva
CapnSp	Capniidae, Capnia sp.	CapnSp	Capniidae, Capnia sp.
CapSch	Capniidae, Capnopsis schilleri	CapSch	Capniidae, Capnopsis schilleri
Cerato	Ceratopogonidae (L)	Cerato	Ceratopogonidae
ChiroL	Chironomidae (L)	Chiron	Chironomidae
ChiroP	Chironomidae (P)		
SipBur	Chloroperlidae, Siphonoperla burmeisteri	SipBur	Chloroperlidae, Siphonoperla burmeisteri
Chryso	Chrysomelidae	Chryso	Chrysomelidae
ColeLW	Coleoptera indet (L)	ColeoW	Coleoptera indet
CurcuL	Curculionidae (L)	Curcul	Curculionidae
CurcuA	Curculionidae (A)		
Diplop	Diplopoda terrestrial	Diplop	Diplopoda terrestrial
DipteW	Diptera indet	DipteW	Diptera indet
Dixida	Dixidae (L)	Dixida	Dixidae
Dryopi	Dryopidae (A)	Dryopi	Dryopidae
Dytisc	Dytiscidae (L)	Dytisc	Dytiscidae
ElmidA	Elmidae (A)	Elmida	Elmidae
ElmidL	Elmidae (L)		
Empidi	Empididae (L)	Empidi	Empididae
EphemW	Ephemerellidae indet	EphemW	Ephemerellidae indet
SerIgn	Ephemerellidae, Serratella ignita	SerIgn	Ephemerellidae, Serratella ignita
Gastro	Gastropoda indet	Gastro	Gastropoda indet
Glossi	Glossiphoniidae	Glossi	Glossiphoniidae
AgapSp	Glossosomatidae, Agapetus sp.	AgapSp	Glossosomatidae, Agapetus sp.
AgaOch	Glossosomatidae, Agapetus ochripes	AgaOch	Glossosomatidae, Agapetus ochripes
SilPal	Goeridae, Silo pallipes	SilPal	Goeridae, Silo pallipes
HepSul	Heptageniidae, Heptagenia sulphurea	HepSul	Heptageniidae, Heptagenia sulphurea
Hetero	Heteroptera	Hetero	Heteroptera
Hydrac	Hydrachnidiae	Hydrac	Hydrachnidiae
Hydrae	Hydraenidae (A)	Hydrae	Hydraenidae
Hydrop	Hydrophilidae (L)	Hydrop	Hydrophilidae
Isopod	Isopoda, Asellus aquaticus	Isopod	Isopoda, Asellus aquaticus
LepHir	Lepidostomatidae, Lepidostoma hirtum	LepHir	Lepidostomatidae, Lepidostoma hirtum
LeuFus	Leuctridae, Leuctra fusca	LeuFus	Leuctridae, Leuctra fusca
LeuHip	Leuctridae, Leuctra hippopus	LeuHip	Leuctridae, Leuctra hippopus
LeuNig	Leuctridae, Leuctra nigra	LeuNig	Leuctridae, Leuctra nigra
LeucSp	Leuctridae, Leuctra sp.	LeucSp	Leuctridae, Leuctra sp.
LimneW	Limnephilidae indet	LimneW	Limnephilidae indet

Original code	Original name	Modified code	Modified name
ChaVil	Limnephilidae, Chaetopteryx villosa	ChaVil	Limnephilidae, Chaetopteryx villosa
HalDig	Limnephilidae, Halesus digitatus	HalDig	Limnephilidae, Halesus digitatus
HalRad	Limnephilidae, Halesus radiatus	HalRad	Limnephilidae, Halesus radiatus
HaleSp	Limnephilidae, Halesus sp.	HaleSp	Limnephilidae, Halesus sp.
PotNig	Limnephilidae, Potamophylax nigricornis	PotNig	Limnephilidae, Potamophylax nigricornis
PotCin	Limnephilidae, Potamophylax cingulatus	PotCin	Limnephilidae, Potamophylax cingulatus
PotLat	Limnephilidae, Potamophylax latipennis	PotLat	Limnephilidae, Potamophylax latipennis
PotaSp	Limnephilidae, Potamophylax sp.	PotaSp	Limnephilidae, Potamophylax sp.
Limoni	Limoniidae (L)	Limoni	Limoniidae
LimAnt	Limoniidae, Antocha sp.	AntoSp	Limoniidae, Antocha sp.
Lymnae	Lymnaeidae	Lymnae	Lymnaeidae
Muscid	Muscidae (L)	Muscid	Muscidae
AmpBor	Nemouridae, Amphinemura borealis	AmpBor	Nemouridae, Amphinemura borealis
AmphSp	Nemouridae, Amphinemura sp.	AmphSp	Nemouridae, Amphinemura sp.
AmpSta	Nemouridae, Amphinemura standfussi	AmpSta	Nemouridae, Amphinemura standfussi
AmpSul	Nemouridae, Amphinemura sulcicollis	AmpSul	Nemouridae, Amphinemura sulcicollis
NemCin	Nemouridae, Nemoura cinerea	NemCin	Nemouridae, Nemoura cinerea
ProMey	Nemouridae, Protonemura meyeri	ProMey	Nemouridae, Protonemura meyeri
NemoSp	Nemouridae, Nemoura sp.	NemoSp	Nemouridae, Nemoura sp.
Oligoc	Oligochaeta	Oligoc	Oligochaeta
Dicran	Pediciidae, Dicranota	Dicran	Pediciidae, Dicranota
PediSp	Pediciidae, Pedicia sp.	PediSp	Pediciidae, Pedicia sp.
IsoDif	Perlodidae, Isoperla difformis	IsoDif	Perlodidae, Isoperla difformis
IsoGra	Perlodidae, Isoperla grammatica	IsoGra	Perlodidae, Isoperla grammatica
IsoSp	Perlodidae, Isoperla sp.	IsopSp	Perlodidae, Isoperla sp.
Physid	Physidae	Physid	Physidae
AncFlu	Planorbidae (form. Ancylidae), Ancylus fluviatilis	AncFlu	Planorbidae, Ancylus fluviatilis
PleCon	Polycentropodidae, Plectrocnemia conspersa	PleCon	Polycentropodidae, Plectrocnemia conspersa
PolFla	Polycentropodidae, Polycentropus flavomaculatus	PolFla	Polycentropodidae, Polycentropus flavomaculatus
Polych	Polychaeta	Polych	Polychaeta
PsychL	Psychodidae (L)	Psycho	Psychodidae
PsychP	Psychodidae (P)		
PsychW	Psychomyiidae indet	PsychW	Psychomyiidae indet
LypRed	Psychomyiidae, Lype reducta	LypRed	Psychomyiidae, Lype reducta
Pyrali	Pyralidae (L)	Pyrali	Pyralidae
RhyNuL	Rhyacophilidae, Rhyacophila nubila (L)	RhyNub	Rhyacophilidae, Rhyacophila nubila
RhyNuP	Rhyacophilidae, Rhyacophila nubila (P)		
Scirti	Scirtidae (L)	Scirti	Scirtidae
SerPer	Sericostomatidae, Sericostoma personatum	SerPer	Sericostomatidae, Sericostoma personatum
SialSp	Sialidae, Sialis sp.	SialSp	Sialidae, Sialis sp.
SimulL	Simuliidae (L)	Simuli	Simuliidae
SimulP	Simuliidae (P)		
BraRis	Taeniopterygidae, Brachyptera risi	BraRis	Taeniopterygidae, Brachyptera risi
TaeNeb	Taeniopterygidae, Taeniopteryx nebulosa	TaeNeb	Taeniopterygidae, Taeniopteryx nebulosa
TipulL	Tipulidae (L)	Tipuli	Tipulidae
TipulP	Tipulidae (P)		
TrichW	Trichoptera indet (L)	Tricho	Trichoptera indet
TrichP	Trichoptera indet (P)		

7.2 Appendix 2 – PCA of all 85 species



7.3 Appendix 3 – ASPT index scores (Miljødirektoratet 2013).

Hovedgrupper	Familier	Verdi
Døgnfluer	Siphlonuridae, Heptageniidae, Leptophlebiidae Ephemerellidae, Potamanthidae, Ephemeridae	10
Steinfluer	Taeniopterygidae, Leuctridae, Capniidae, Perlodidae, Perlidae, Chloroperlidae	10
Teger	Aphelocheridae	10
Vårfluer	Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae	10
Kreps	Astacidae	8
Øyenstikkere	Lestidae, Agriidae, Gomphidae, Cordulegasteridae, Aeshnidae, Corduliidae, Libelluiidae	8
Vårfluer	Philopotamidae	8
Døgnfluer	Caenidae	7
Steinfluer	Nemouridae	7
Vårfluer	Rhyacophilidae, Polycentropidae, Limnephilidae	7
Snegler	Neritidae, Viviparidae, Ancylidae	6
Vårfluer	Hydroptilidae	6
Muslinger	Unionidae	6
Krepsdyr	Corophiidae, Gammaridae	6
Øyenstikkere	Platycnemididae, Coenagriidae	6
Teger	Mesoveliidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Notonectidae, Pleidae, Corixidae	5
Biller	Haliplidae, Hygrobiidae, Dytiscidae, Gyrinidae, Hydrophilidae, Clambidae, Helodidae, Dryopidae, Elmidae, Chrysomelidae, Curculionidae	5
Vårfluer	Hydropsychidae	5
Stankelbein/Knott	Tipulidae, Simuliidae	5
Flatormer	Planariidae, Dendrocoelidae	5
Døgnfluer	Baetidae	4
Mudderfluer	Sialidae	4
Igler	Piscicolidae	4
Snegler	Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae	3
Småmuslinger	Sphaeriidae	3
Igler	Glossiphoniidae, Hirudidae, Erpobdellidae	3
Ferskvannsasell	Asellidae	3
Fjærmygg	Chironomidae	2
Fåbørstemark	Oligochaeta (hele klassen)	1

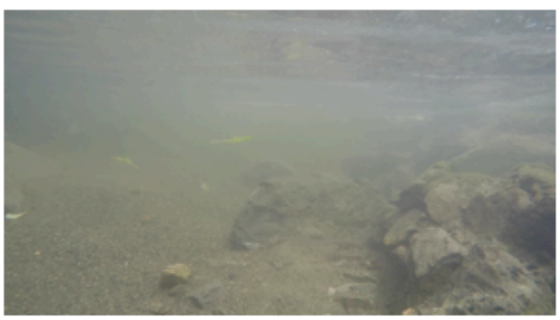
7.4 Appendix 4 – Photos from all stations autumn 2015

Vigga upstream (A15VU)

Vøien (A15Vøi)

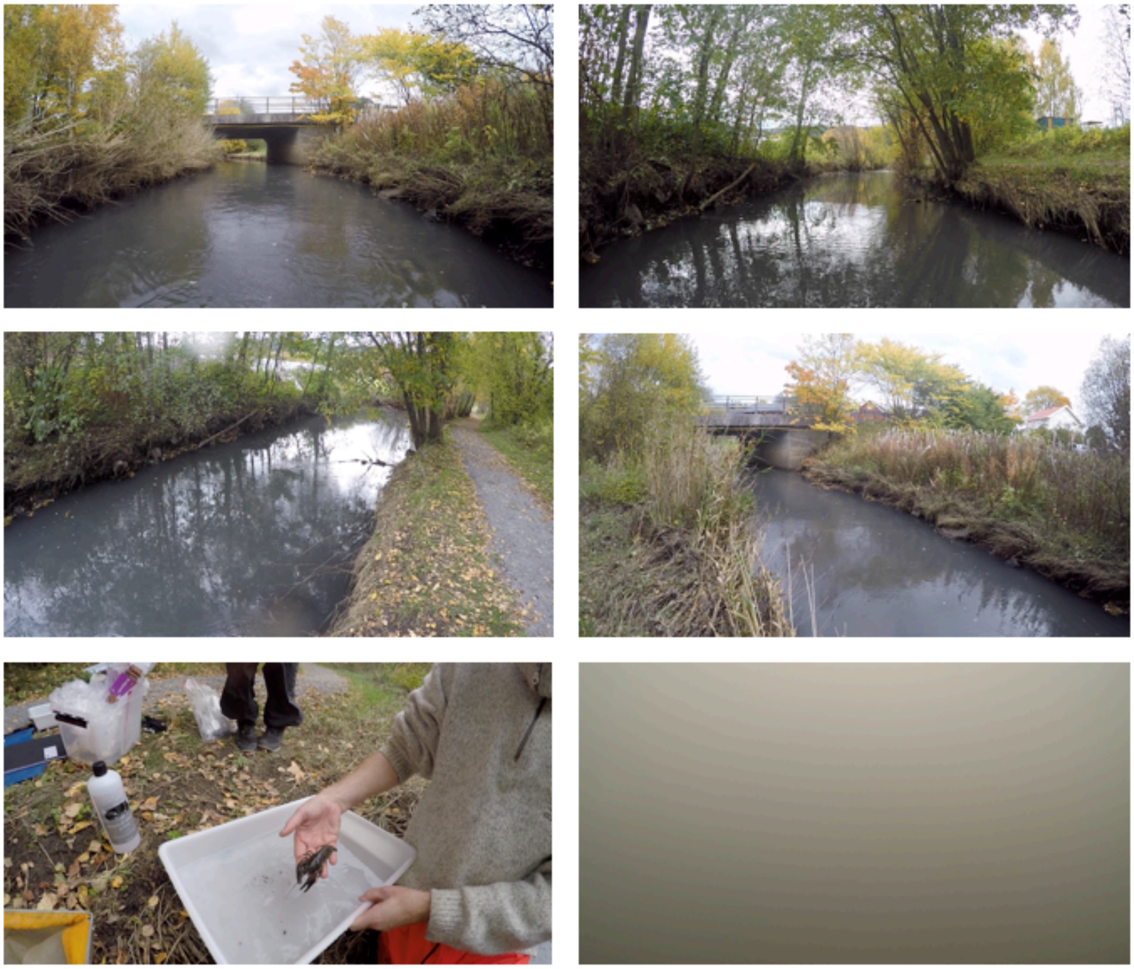







School (A15Sch)

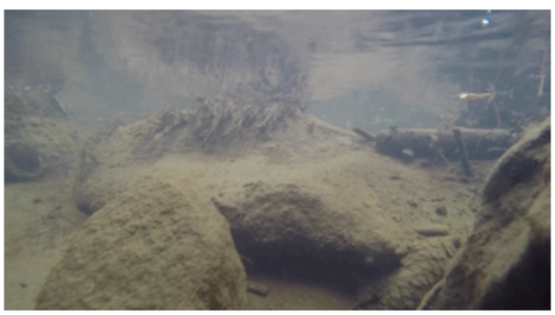
Vigga downstream (A15VDS)



Nordtangen (A15Nor)

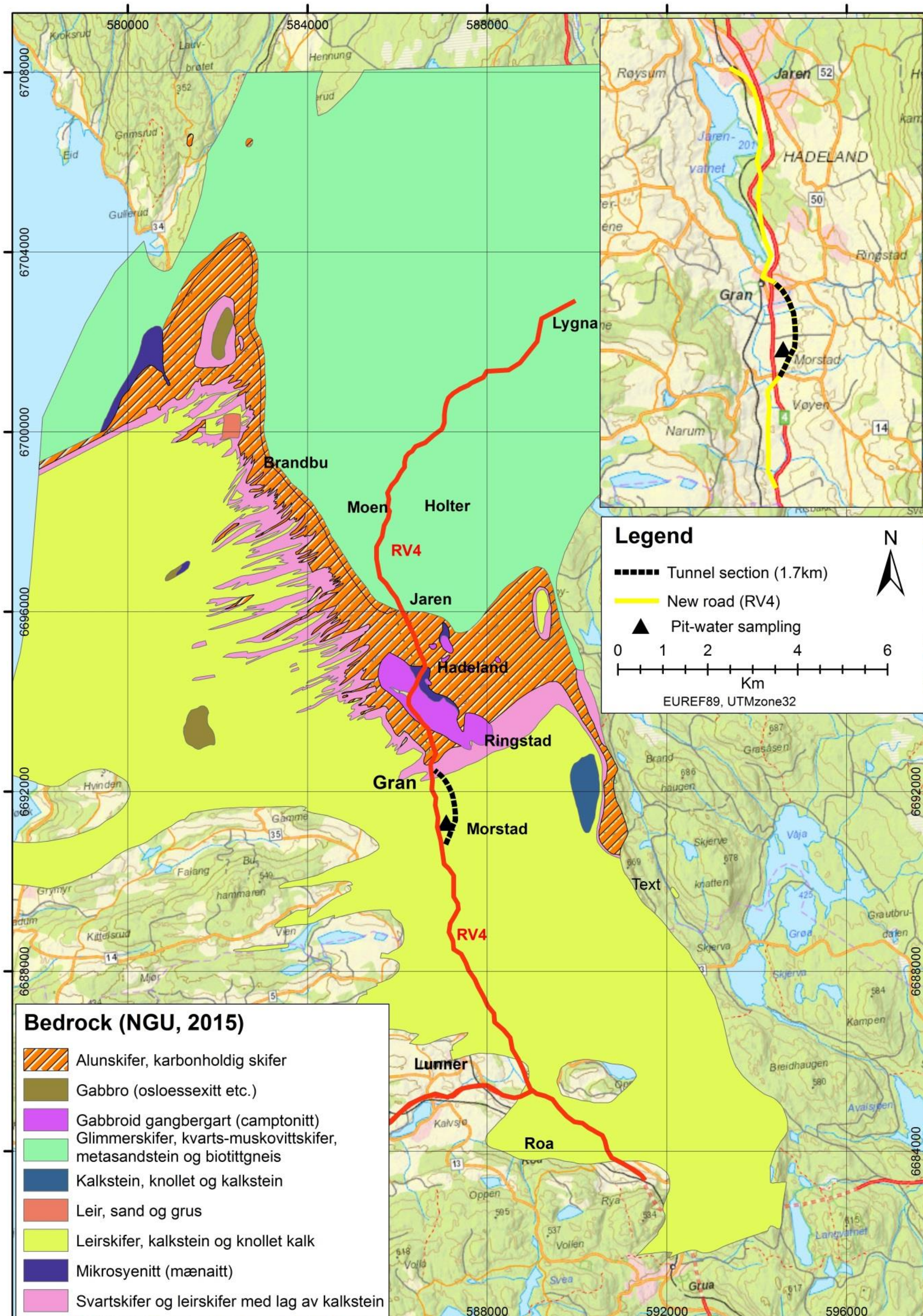







Vigga upstream 2 (A15VU2)

7.5 Appendix 5 – The geology of the Gran area (Ahmad 2015)



7.6 Appendix 6 – Raw data macroinvertebrates

Sample ID	Acari	Aranea	AlaMut	BaeNig	BaeRho	BaetSp	CenLut	Bivalv	CapnSp	CapSch	Cerato	Chiron	SipBur
S13VU-1	0	0	16	0	40	0	0	0	0	0	0	132	0
S13VU-2	0	0	40	0	36	0	0	0	0	0	0	60	0
S13VU-3	0	0	0	0	48	0	0	0	0	0	0	68	0
S13VDS	0	0	28	0	48	0	0	0	0	0	0	148	0
S13Sch-1	0	0	16	0	40	0	0	0	0	0	0	96	0
S13Sch-2	0	0	8	0	112	0	0	0	0	0	0	104	0
S13Vøi-1	0	0	32	4	284	0	0	0	0	0	0	4	0
S13Vøi-2	0	0	96	0	616	0	0	0	0	0	0	28	8
S13Vøi-3	0	0	60	0	1124	0	0	0	0	0	0	0	12
A13Vøi-1	0	8	0	0	896	0	0	0	0	0	0	8	36
A13Vøi-2	4	12	0	0	368	0	0	0	0	0	0	4	12
A13Vøi-3	0	12	0	0	324	0	0	0	0	0	0	8	8
A13VU-1	0	0	0	0	56	0	0	0	0	0	0	0	0
A13VU-2	0	0	8	4	36	0	0	0	0	0	0	0	0
A13VU-3	0	4	0	16	80	0	0	1	0	0	0	8	8
A13VDS-1	0	0	0	52	4	0	0	0	0	0	0	8	20
A13VDS-2	0	0	8	60	4	0	0	0	0	0	4	16	4
A13VDS-3	0	0	0	8	0	0	0	0	0	0	0	8	4
S15VDS-1	0	0	4	0	20	0	0	0	0	0	0	56	0
S15VDS-2	0	0	8	0	32	0	0	0	0	0	0	164	0
S15VDS-3	0	0	8	0	48	0	0	0	0	0	0	132	0
S15Vøi-1	0	0	76	8	452	0	0	0	0	0	4	68	4
S15Vøi-2	0	0	56	0	64	0	0	0	0	0	0	36	0
S15Vøi-3	0	4	216	0	736	0	0	0	0	0	0	152	0
S15Nor-1	0	0	0	0	0	0	0	0	0	0	0	100	0
S15Nor-2	0	0	0	0	4	0	0	0	0	0	0	112	0
S15Nor-3	0	0	0	0	0	0	0	0	0	0	0	116	0
S15VU-1	0	0	8	0	112	0	0	0	0	0	4	176	0
S15VU-2	0	0	16	0	52	0	0	0	0	0	0	176	0
S15VU-3	0	0	12	0	116	0	0	0	0	0	0	117	0
S15VU2-1	0	0	4	0	136	0	0	0	0	0	0	120	0
S15VU2-2	0	0	0	0	184	4	0	0	0	0	0	116	0
S15VU2-3	0	0	0	0	260	0	0	0	0	0	0	220	0
S15Sch-1	0	0	88	0	116	0	0	4	0	0	4	180	4
S15Sch-2	0	0	116	0	156	0	0	0	0	0	0	172	12
S15Sch-3	0	0	56	8	68	0	0	0	0	0	0	776	12
A15VU2-1	0	4	0	20	340	0	0	0	0	0	4	44	0
A15VU2-2	0	4	0	16	600	0	0	0	12	0	0	28	0
A15VU2-3	0	0	0	44	540	4	0	0	4	0	0	20	0
A15Nor-1	0	0	0	0	132	0	0	0	0	0	1	80	0
A15Nor-2	0	0	0	0	48	0	0	0	0	0	4	88	0
A15Nor-3	0	0	4	4	20	0	0	0	0	1	12	100	0
A15VU-1	0	0	0	48	56	0	0	0	0	0	4	32	0
A15VU-2	0	0	0	36	108	0	0	0	0	0	12	60	0
A15VU-3	0	0	0	16	68	0	0	0	0	0	0	24	0
A15VDS-1	0	0	0	8	140	0	8	0	0	4	0	12	28
A15VDS-2	0	0	0	20	108	0	0	0	0	0	0	16	20
A15VDS-3	0	0	0	40	268	0	28	0	0	0	4	16	16
A15Sch-1	4	0	160	303	580	0	0	0	0	4	4	40	32
A15Sch-2	0	0	16	16	408	0	0	0	0	0	0	4	8
A15Sch-3	0	0	64	0	296	0	0	0	0	0	0	4	12
A15Vøi-1	4	0	40	12	276	8	0	0	0	12	0	24	4
A15Vøi-2	0	4	104	172	1220	0	0	0	0	20	0	36	20
A15Vøi-3	0	24	4	76	632	0	0	0	0	0	0	40	0

Sample ID	Chryso	ColeoW	Curcul	Diplop	DipteW	Dixida	Dryopi	Dytisc	Elmida	Empidi	EphemW	Serign	Gastro
S13VU-1	0	0	0	0	0	0	0	8	540	0	0	4	0
S13VU-2	0	0	0	0	0	0	0	0	500	8	12	0	0
S13VU-3	0	0	0	0	0	0	0	0	188	0	0	4	0
S13VDS	0	0	0	0	0	0	0	0	100	0	0	0	0
S13Sch-1	0	0	0	0	0	0	0	0	4	0	0	0	0
S13Sch-2	0	0	0	0	0	0	0	0	4	0	0	0	0
S13Vøi-1	0	0	0	0	0	0	0	0	4	0	0	0	0
S13Vøi-2	0	0	0	0	0	0	0	0	0	0	0	0	0
S13Vøi-3	0	4	0	0	0	0	0	0	4	0	0	0	4
A13Vøi-1	0	0	0	0	0	12	0	0	64	0	0	0	4
A13Vøi-2	0	1	0	0	0	0	0	0	40	4	0	0	4
A13Vøi-3	0	0	4	0	0	60	4	4	24	0	0	0	0
A13VU-1	0	0	0	0	0	0	0	0	284	0	0	0	0
A13VU-2	0	0	0	0	0	0	0	1	372	0	0	0	0
A13VU-3	0	0	0	0	0	0	0	0	268	0	0	0	0
A13VDS-1	0	0	0	0	0	0	0	4	68	0	0	0	0
A13VDS-2	0	0	0	0	0	0	0	4	160	0	0	4	0
A13VDS-3	0	0	0	0	0	0	0	0	68	0	0	0	0
S15VDS-1	0	0	0	0	0	0	0	0	36	0	0	0	1
S15VDS-2	4	0	0	0	0	0	0	0	132	0	0	0	0
S15VDS-3	0	0	0	0	0	0	0	0	180	4	0	0	0
S15Vøi-1	0	0	0	0	0	0	0	0	8	4	0	0	0
S15Vøi-2	0	0	0	0	0	0	0	0	4	0	0	0	1
S15Vøi-3	0	0	0	0	0	0	0	0	32	4	0	0	4
S15Nor-1	0	0	0	0	0	0	0	0	0	0	0	0	0
S15Nor-2	0	0	0	0	0	0	0	0	0	0	0	0	0
S15Nor-3	0	4	0	0	0	0	0	0	0	0	0	0	0
S15VU-1	0	0	0	0	0	0	0	0	472	8	0	0	0
S15VU-2	0	0	0	0	4	0	0	0	300	1	0	0	0
S15VU-3	0	0	0	0	0	0	0	0	508	0	0	0	1
S15VU2-1	0	0	0	0	0	0	0	0	176	0	0	0	0
S15VU2-2	0	0	0	0	4	0	0	0	120	4	0	0	0
S15VU2-3	0	0	0	0	0	0	0	0	192	16	0	0	0
S15Sch-1	0	0	4	0	0	0	0	0	0	0	0	0	4
S15Sch-2	0	0	0	0	0	0	0	1	0	0	0	0	0
S15Sch-3	0	0	0	0	1	4	0	0	0	0	0	0	4
A15VU2-1	0	0	0	0	0	0	0	0	13	0	0	0	4
A15VU2-2	0	0	0	4	0	0	0	0	52	0	0	0	0
A15VU2-3	0	0	0	0	0	0	0	0	80	0	0	0	0
A15Nor-1	0	0	0	0	0	0	0	8	0	0	0	0	4
A15Nor-2	0	0	0	1	0	0	0	0	0	0	0	0	0
A15Nor-3	0	0	0	0	0	0	0	0	0	0	0	0	0
A15VU-1	0	0	0	0	0	0	0	0	376	0	0	0	0
A15VU-2	0	0	0	0	0	0	0	0	556	8	0	4	0
A15VU-3	0	0	0	0	0	0	0	2	120	0	0	0	2
A15VDS-1	0	0	0	0	0	0	0	0	56	0	0	0	0
A15VDS-2	0	0	0	0	0	0	0	0	88	0	0	4	0
A15VDS-3	0	0	0	0	0	0	0	4	116	0	0	0	0
A15Sch-1	0	0	0	0	0	16	0	0	0	0	0	0	0
A15Sch-2	0	0	0	0	0	0	0	0	0	0	0	0	0
A15Sch-3	0	0	0	0	0	4	0	0	0	0	0	0	0
A15Vøi-1	0	0	0	0	0	0	0	0	8	1	0	0	0
A15Vøi-2	0	0	0	0	0	24	0	0	16	0	0	0	0
A15Vøi-3	4	0	0	0	0	4	0	0	4	0	0	0	3

Sample ID	Glossi	AgapSp	AgaOch	SilPal	HepSul	Hetero	Hydrac	Hydrae	Hydrop	Isopod	LepHir	LeuFus	LeuHip
S13VU-1	0	0	0	4	12	0	40	36	0	0	0	40	0
S13VU-2	0	0	0	8	1	0	40	96	0	0	0	0	0
S13VU-3	0	0	0	8	8	0	40	4	0	0	0	0	8
S13VDS	0	0	0	4	0	0	16	60	0	0	0	16	0
S13Sch-1	0	0	0	0	0	0	0	44	0	4	0	0	0
S13Sch-2	0	0	0	4	0	4	0	28	0	0	0	0	0
S13Vøi-1	0	0	0	4	0	0	0	80	0	0	0	0	0
S13Vøi-2	0	0	0	0	0	0	4	136	0	1	0	0	0
S13Vøi-3	0	0	0	0	0	4	0	300	0	0	0	0	0
A13Vøi-1	0	0	0	0	0	12	0	1540	0	0	0	72	0
A13Vøi-2	0	0	0	0	0	0	4	116	0	0	0	0	0
A13Vøi-3	0	0	0	0	0	12	0	252	0	0	0	0	16
A13VU-1	0	0	0	4	0	0	8	252	0	0	0	0	0
A13VU-2	0	0	0	0	4	0	4	272	0	0	0	0	0
A13VU-3	0	0	0	4	8	0	56	152	0	0	0	0	0
A13VDS-1	0	0	0	0	0	0	8	24	0	0	0	68	0
A13VDS-2	0	0	0	0	12	4	52	32	8	0	0	16	0
A13VDS-3	0	0	0	0	1	0	0	4	0	0	0	4	0
S15VDS-1	0	0	0	0	0	0	0	0	0	0	0	0	0
S15VDS-2	0	0	0	4	0	1	0	4	0	0	0	0	0
S15VDS-3	1	0	0	0	0	0	0	4	0	0	0	0	0
S15Vøi-1	0	0	0	0	0	4	0	108	0	0	0	0	8
S15Vøi-2	0	0	0	0	0	0	0	36	0	0	0	0	0
S15Vøi-3	0	0	0	0	0	4	4	164	0	0	0	4	0
S15Nor-1	0	0	0	0	0	0	0	0	4	0	0	0	0
S15Nor-2	0	0	0	0	0	4	0	0	0	0	0	0	0
S15Nor-3	0	0	0	0	0	0	0	0	0	0	0	0	0
S15VU-1	0	0	0	8	1	4	24	60	0	0	0	0	0
S15VU-2	0	0	0	0	0	4	4	28	0	0	0	0	0
S15VU-3	0	0	0	0	0	20	24	52	0	0	0	0	0
S15VU2-1	0	0	0	0	0	0	68	40	0	0	0	0	4
S15VU2-2	0	0	0	0	0	0	20	48	0	0	0	0	0
S15VU2-3	0	0	0	0	0	8	16	32	0	0	0	4	0
S15Sch-1	0	0	0	4	0	0	0	92	0	0	0	0	0
S15Sch-2	0	0	0	8	0	4	0	140	0	0	0	0	0
S15Sch-3	0	0	0	12	0	4	0	116	0	0	0	0	0
A15VU2-1	0	0	4	0	0	0	12	1	0	0	0	0	8
A15VU2-2	0	0	0	0	0	0	16	28	0	0	0	0	16
A15VU2-3	0	0	0	0	0	0	20	56	0	0	1	0	0
A15Nor-1	0	0	0	0	0	0	0	0	0	0	0	0	0
A15Nor-2	0	0	0	0	0	0	0	0	0	0	0	0	0
A15Nor-3	0	0	0	0	0	0	0	4	0	0	0	0	0
A15VU-1	0	0	0	0	0	0	8	56	0	0	0	0	12
A15VU-2	0	4	0	0	0	0	88	148	0	0	0	0	12
A15VU-3	0	0	0	4	0	1	40	68	0	0	0	0	0
A15VDS-1	0	0	0	4	0	0	12	16	0	0	0	0	4
A15VDS-2	0	0	0	0	0	0	0	4	0	0	0	0	0
A15VDS-3	0	0	0	0	0	0	12	16	0	0	0	0	0
A15Sch-1	0	0	0	4	0	0	4	116	0	0	0	0	28
A15Sch-2	0	0	0	4	0	0	0	36	0	0	0	0	4
A15Sch-3	0	0	0	0	0	0	0	20	0	0	0	8	0
A15Vøi-1	0	0	0	0	0	4	4	16	0	0	0	0	48
A15Vøi-2	0	0	0	0	0	0	16	168	0	0	0	0	44
A15Vøi-3	0	0	0	0	0	0	4	40	0	0	0	0	12

Sample ID	LeuNig	LeucSp	LimneW	ChaVil	HalDig	HalRad	HaleSp	PotNig	PotCin	PotLat	PotaSp	Limoni	AntoSp
S13VU-1	0	0	0	12	0	0	0	0	0	0	0	4	0
S13VU-2	0	52	0	4	0	0	0	0	0	0	0	0	0
S13VU-3	0	0	0	8	0	0	0	0	0	0	0	1	0
S13VDS	0	0	0	0	0	2	0	4	0	0	0	4	0
S13Sch-1	0	0	0	4	0	1	0	0	0	0	0	0	0
S13Sch-2	0	0	0	0	0	1	0	1	0	0	0	0	0
S13Vøi-1	0	0	0	0	0	0	0	0	0	0	1	0	0
S13Vøi-2	0	0	4	0	0	1	0	0	0	1	0	0	0
S13Vøi-3	0	0	0	0	0	0	0	0	0	0	0	0	0
A13Vøi-1	12	0	8	0	0	0	0	0	0	0	0	0	0
A13Vøi-2	20	0	8	0	0	0	0	0	0	0	0	4	0
A13Vøi-3	4	0	4	0	0	0	0	0	0	0	0	1	0
A13VU-1	0	8	0	0	0	0	0	0	0	0	0	0	0
A13VU-2	0	0	0	0	0	0	0	0	0	0	0	1	0
A13VU-3	0	0	0	0	0	0	0	0	0	0	0	0	0
A13VDS-1	0	1	0	0	0	0	0	0	0	0	0	0	0
A13VDS-2	0	0	0	0	0	0	0	0	0	0	0	0	0
A13VDS-3	0	0	0	0	0	0	0	0	0	0	0	0	0
S15VDS-1	0	0	0	0	0	0	0	0	0	0	0	1	0
S15VDS-2	0	0	0	0	0	8	0	0	0	0	0	0	0
S15VDS-3	0	4	0	0	0	1	0	0	0	0	0	0	0
S15Vøi-1	0	0	0	1	0	0	0	0	4	0	0	0	0
S15Vøi-2	0	0	0	8	0	2	0	0	0	0	0	0	0
S15Vøi-3	0	0	0	4	0	4	0	0	0	0	0	0	0
S15Nor-1	0	0	0	0	0	1	0	0	0	0	0	0	0
S15Nor-2	0	0	0	0	0	0	0	0	0	0	0	0	0
S15Nor-3	0	0	0	0	0	0	0	0	0	0	0	0	0
S15VU-1	0	0	0	0	1	0	0	0	0	0	0	4	0
S15VU-2	0	0	0	0	1	0	0	0	0	0	0	8	1
S15VU-3	0	4	0	0	0	1	0	0	0	0	0	0	0
S15VU2-1	0	0	4	0	0	0	0	0	0	0	0	8	0
S15VU2-2	0	0	0	0	0	0	0	0	0	0	0	0	0
S15VU2-3	4	0	0	0	0	0	0	0	0	0	0	0	0
S15Sch-1	0	0	0	4	0	1	0	0	0	2	0	0	0
S15Sch-2	0	0	0	24	1	0	0	0	0	0	0	0	0
S15Sch-3	0	8	0	1	0	4	0	0	0	0	0	0	0
A15VU2-1	4	0	1	0	0	0	0	0	0	0	0	0	0
A15VU2-2	12	0	1	0	0	0	0	0	0	0	0	0	0
A15VU2-3	0	12	4	0	0	0	0	0	0	0	0	0	0
A15Nor-1	4	0	0	0	0	0	0	0	0	0	0	0	0
A15Nor-2	0	0	16	0	0	0	0	0	0	0	0	0	0
A15Nor-3	0	0	12	0	0	0	0	0	0	0	0	4	0
A15VU-1	0	0	4	0	0	0	0	0	0	0	0	0	0
A15VU-2	0	0	0	0	0	0	0	0	0	0	0	0	4
A15VU-3	0	4	0	0	0	0	8	0	0	0	0	0	0
A15VDS-1	0	0	8	0	0	0	0	0	0	0	0	4	0
A15VDS-2	0	4	0	0	4	0	0	0	0	0	0	0	0
A15VDS-3	0	0	4	0	0	0	0	0	0	4	0	0	4
A15Sch-1	4	0	12	0	0	0	0	0	0	4	0	1	0
A15Sch-2	0	0	0	0	0	0	0	0	0	0	0	0	0
A15Sch-3	0	0	4	0	0	0	0	0	0	0	0	0	0
A15Vøi-1	12	0	36	0	0	0	0	0	0	0	0	4	0
A15Vøi-2	60	0	64	0	0	0	0	0	0	0	8	1	0
A15Vøi-3	0	0	4	0	0	0	0	0	0	0	0	48	0

Sample ID	Lymnae	Muscid	AmpBor	AmphSp	AmpSta	AmpSul	NemCin	ProMey	NemoSp	Oligoc	Dicran	PediSp
S13VU-1	0	0	0	0	0	0	0	0	0	4	8	0
S13VU-2	0	0	0	0	0	0	0	0	0	8	4	0
S13VU-3	0	0	0	0	0	0	0	0	0	4	4	0
S13VDS	0	0	8	4	0	0	0	0	0	12	8	0
S13Sch-1	0	0	0	0	20	0	0	0	0	0	24	0
S13Sch-2	0	0	0	0	44	0	0	0	0	24	8	0
S13Vøi-1	0	0	0	0	12	0	0	0	0	0	4	0
S13Vøi-2	0	0	0	0	84	0	0	0	0	20	4	0
S13Vøi-3	0	0	0	0	40	0	0	0	0	1	8	0
A13Vøi-1	0	0	0	0	0	0	0	4	0	36	28	0
A13Vøi-2	0	0	0	0	0	0	8	8	0	112	0	0
A13Vøi-3	0	0	0	0	0	0	0	0	0	4	8	0
A13VU-1	0	0	0	0	0	0	0	140	0	44	8	0
A13VU-2	0	0	0	0	0	0	16	100	0	1	8	0
A13VU-3	0	0	4	0	0	0	0	36	0	36	8	0
A13VDS-1	0	0	0	0	0	0	0	4	0	0	0	0
A13VDS-2	0	0	0	0	8	0	0	8	0	12	8	0
A13VDS-3	0	0	0	0	0	0	1	0	0	1	0	0
S15VDS-1	0	0	0	0	0	0	0	0	0	8	12	0
S15VDS-2	0	0	0	0	0	0	0	0	0	4	4	0
S15VDS-3	0	0	0	0	0	0	0	0	0	12	8	0
S15Vøi-1	0	0	0	4	0	0	0	0	0	28	4	0
S15Vøi-2	0	0	0	4	0	0	0	0	0	32	64	0
S15Vøi-3	0	0	0	0	20	0	0	0	0	32	28	0
S15Nor-1	0	0	0	0	12	0	0	0	0	8	4	0
S15Nor-2	0	0	0	0	100	0	0	0	0	16	4	0
S15Nor-3	0	1	0	0	68	0	4	0	0	12	1	0
S15VU-1	0	0	0	0	0	1	0	0	0	20	1	0
S15VU-2	0	0	0	8	0	0	0	0	0	2	4	0
S15VU-3	0	0	0	0	0	0	0	0	0	4	0	0
S15VU2-1	0	0	0	0	0	0	0	0	0	8	28	0
S15VU2-2	0	0	0	0	0	0	0	0	0	20	12	0
S15VU2-3	16	0	0	0	0	0	0	0	0	4	4	0
S15Sch-1	0	0	0	0	8	0	0	0	0	8	36	0
S15Sch-2	8	0	0	0	8	0	0	0	0	12	8	0
S15Sch-3	0	0	0	0	20	0	0	0	0	16	20	0
A15VU2-1	0	0	0	28	0	0	0	4	0	4	8	0
A15VU2-2	0	0	0	32	0	0	0	4	0	16	4	0
A15VU2-3	0	0	8	0	0	8	0	0	0	8	8	0
A15Nor-1	0	0	0	0	0	0	68	0	0	24	16	0
A15Nor-2	0	0	0	0	0	0	0	0	20	28	36	1
A15Nor-3	0	0	0	0	0	0	0	0	36	28	48	0
A15VU-1	0	0	4	0	0	0	0	0	0	0	8	0
A15VU-2	0	0	4	12	0	0	0	0	0	12	8	0
A15VU-3	0	0	0	0	0	0	0	1	0	8	1	0
A15VDS-1	0	0	0	0	0	4	4	0	0	16	3	0
A15VDS-2	0	0	0	0	0	12	4	0	0	12	2	0
A15VDS-3	0	0	0	0	0	0	4	0	0	28	4	0
A15Sch-1	0	0	0	0	0	56	24	40	0	20	24	0
A15Sch-2	0	0	20	0	0	20	0	8	0	4	1	0
A15Sch-3	0	0	0	16	0	0	0	4	0	24	8	0
A15Vøi-1	0	0	0	0	0	4	0	16	0	16	0	0
A15Vøi-2	0	0	0	0	0	16	4	28	0	16	4	0
A15Vøi-3	0	0	0	4	0	0	0	44	0	44	16	0

Sample ID	IsoDif	IsoGra	IsopSp	Physid	AncFlu	PleCon	PolFla	Polych	Psycho	PsychW	LypRed	Pyrali	RhyNub	Scirti
S13VU-1	0	0	0	0	0	0	0	0	0	0	0	0	16	0
S13VU-2	0	0	0	0	0	0	0	0	0	0	0	0	39	0
S13VU-3	0	0	0	0	4	0	0	0	0	0	0	0	1	0
S13VDS	0	0	0	0	0	0	0	0	0	0	0	0	20	0
S13Sch-1	0	4	0	0	0	0	0	0	0	0	0	0	8	0
S13Sch-2	0	0	0	0	0	0	0	0	0	0	0	0	8	0
S13Vøi-1	0	0	0	0	0	0	0	0	0	0	0	0	4	0
S13Vøi-2	0	4	0	0	0	4	0	0	0	0	0	0	9	0
S13Vøi-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A13Vøi-1	8	0	0	0	0	0	0	0	160	0	0	0	112	0
A13Vøi-2	0	12	0	0	0	0	0	0	36	0	0	0	40	4
A13Vøi-3	4	0	0	0	0	0	0	0	156	4	0	0	12	0
A13VU-1	0	28	0	0	0	0	0	0	8	0	0	0	28	0
A13VU-2	0	0	0	0	0	0	0	0	4	0	0	0	16	0
A13VU-3	16	0	0	0	0	0	0	0	12	0	0	0	24	0
A13VDS-1	1	0	8	0	0	0	1	0	0	0	0	0	0	0
A13VDS-2	0	0	64	0	0	0	1	0	12	0	0	0	8	0
A13VDS-3	0	0	0	0	0	0	4	0	1	0	0	0	3	0
S15VDS-1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S15VDS-2	0	0	0	0	0	4	1	0	0	0	0	0	0	0
S15VDS-3	0	0	0	0	1	0	0	0	0	0	0	0	1	0
S15Vøi-1	0	0	0	0	0	0	0	0	12	0	0	0	4	0
S15Vøi-2	0	1	0	0	0	4	0	0	12	0	0	0	1	0
S15Vøi-3	0	4	0	0	0	4	0	0	4	0	0	0	0	0
S15Nor-1	0	0	0	0	0	0	0	1	0	0	0	4	0	0
S15Nor-2	0	0	0	0	0	0	0	0	4	0	0	0	0	0
S15Nor-3	0	0	0	0	0	0	0	0	4	0	0	0	0	0
S15VU-1	0	0	0	0	0	0	0	0	0	0	0	0	16	0
S15VU-2	0	0	0	0	0	0	0	0	0	0	0	0	8	0
S15VU-3	0	0	0	0	0	0	0	0	0	0	0	0	20	0
S15VU2-1	0	0	0	0	0	0	0	0	0	0	0	0	4	0
S15VU2-2	0	0	0	0	0	0	0	0	0	0	0	0	8	0
S15VU2-3	0	0	0	0	0	0	0	0	0	0	0	0	8	0
S15Sch-1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
S15Sch-2	0	0	0	0	0	0	0	0	4	0	0	0	12	0
S15Sch-3	0	4	0	0	0	0	0	0	0	0	0	0	4	0
A15VU2-1	0	0	0	0	0	0	0	0	16	0	0	0	8	0
A15VU2-2	4	0	0	0	0	0	0	0	80	0	0	0	16	0
A15VU2-3	0	0	0	0	0	0	0	0	20	0	0	0	24	0
A15Nor-1	0	0	0	0	0	0	0	0	4	0	0	0	0	0
A15Nor-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A15Nor-3	0	0	0	0	0	0	0	0	4	0	0	0	0	0
A15VU-1	0	0	0	0	0	0	0	0	16	0	0	0	16	0
A15VU-2	0	0	0	0	0	0	1	0	16	0	1	0	28	0
A15VU-3	8	0	0	0	0	0	0	0	0	0	0	0	4	0
A15VDS-1	4	0	0	0	0	0	0	0	8	0	0	0	4	0
A15VDS-2	8	0	0	0	0	0	0	0	0	0	0	0	12	0
A15VDS-3	4	0	0	0	0	0	0	0	8	0	0	0	2	0
A15Sch-1	68	0	12	0	0	0	0	0	396	0	0	0	24	0
A15Sch-2	24	0	0	0	0	0	0	0	28	0	0	0	12	0
A15Sch-3	16	0	0	0	0	0	0	0	60	0	0	0	12	0
A15Vøi-1	4	0	0	0	0	0	0	0	160	0	0	0	4	0
A15Vøi-2	48	0	0	0	0	0	0	0	296	0	0	0	32	0
A15Vøi-3	20	0	0	0	0	1	0	0	248	0	0	0	20	0

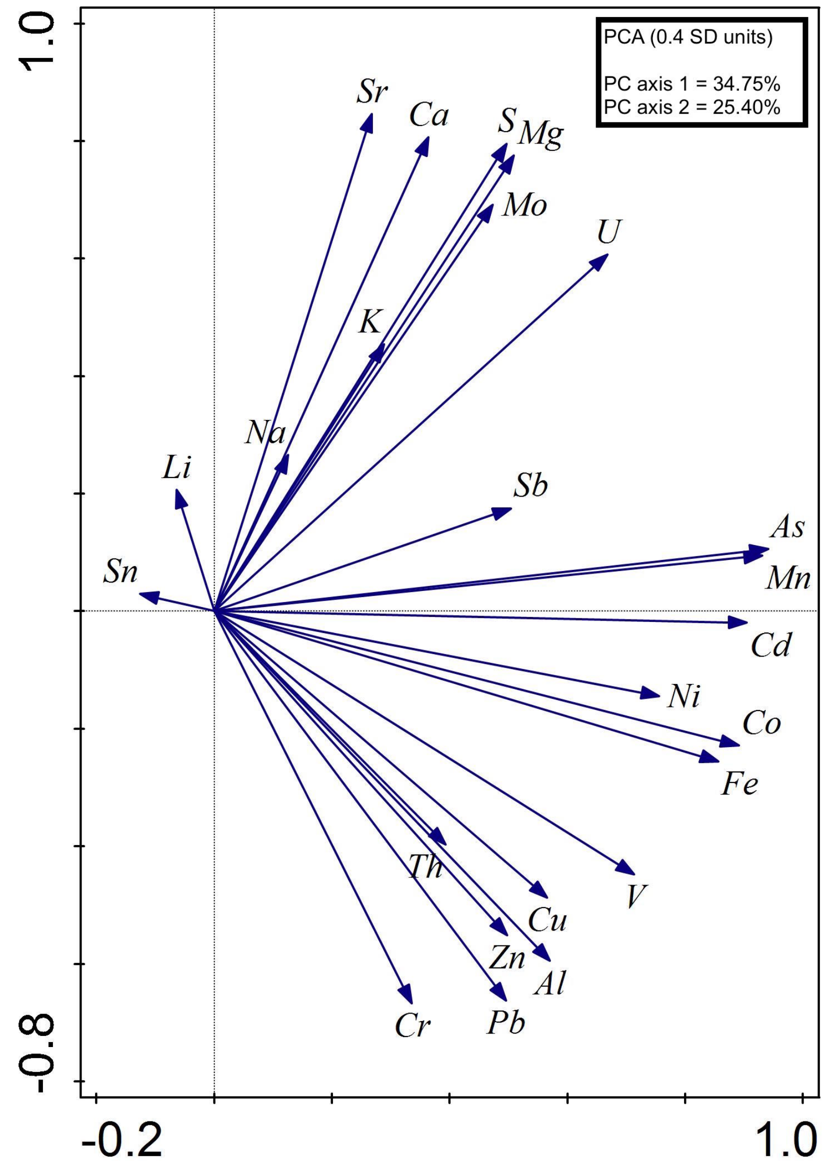
7.7 Appendix 7 – Raw data mayfly metals (red = ½ of detection limit, green = ½ of quantification limit)

Sample ID	Al	S	Ca	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	Th	U
S13VU-1	320,00	760,00	310,00	38,00	330,00	0,69	1,10	4,40	63,00	0,36	0,35	0,17	0,05	0,04
S13VU-2	320,00	670,00	300,00	27,00	320,00	0,46	0,92	3,30	40,00	0,31	0,26	0,19	0,04	0,04
S13VU-3	420,00	630,00	440,00	43,00	430,00	0,56	1,60	3,20	32,00	0,34	0,23	0,29	0,08	0,07
S13Vøi-1	210,00	1100,00	770,00	17,00	210,00	0,55	0,83	4,60	24,00	0,27	0,35	0,09	0,03	0,04
S13Vøi-2	280,00	950,00	470,00	22,00	290,00	0,53	0,78	5,00	27,00	0,18	0,35	0,13	0,04	0,03
S13Vøi-3	640,00	880,00	480,00	20,00	460,00	0,64	1,30	4,20	22,00	0,21	0,31	0,21	0,08	0,04
S13Vøi-4	280,00	1300,00	740,00	22,00	300,00	0,67	0,85	6,20	31,00	0,21	0,46	0,12	0,04	0,03
S13Vøi-5	130,00	790,00	540,00	22,00	150,00	0,56	0,52	3,70	19,00	0,15	0,32	0,06	0,02	0,01
S13Vøi-6	5900,00	1000,00	800,00	64,00	4400,00	1,50	4,10	6,40	34,00	0,57	0,41	0,71	0,69	0,27
S13Sch-1	290,00	1000,00	530,00	22,00	320,00	0,65	0,91	4,40	26,00	0,27	0,29	0,15	0,04	0,04
S13Sch-2	990,00	1200,00	730,00	44,00	840,00	0,93	1,70	5,70	34,00	0,54	0,38	0,43	0,13	0,11
S13Sch-3	1200,00	880,00	670,00	45,00	850,00	0,83	1,80	5,30	30,00	0,33	0,35	0,32	0,14	0,10
S13VDS-1	140,00	690,00	230,00	17,00	160,00	0,42	0,56	2,60	27,00	0,37	0,16	0,08	0,03	0,03
S13VDS-2	180,00	760,00	670,00	26,00	180,00	0,54	0,74	2,80	27,00	0,38	0,27	0,12	0,15	0,03
S13VDS-3	3200,00	990,00	680,00	110,00	2900,00	1,80	7,00	5,90	69,00	1,00	0,34	0,79	0,33	0,15
S13Nor-1	870,00	1000,00	880,00	330,00	900,00	0,74	1,60	8,50	46,00	0,56	2,60	0,37	0,17	0,24
S13Nor-2	430,00	700,00	440,00	110,00	430,00	0,38	0,85	5,30	28,00	0,37	1,50	0,19	0,09	0,10
S13Nor-3	510,00	800,00	640,00	150,00	540,00	0,45	1,10	6,60	35,00	0,40	1,50	0,24	0,10	0,11
A13VU	54,00	340,00	120,00	12,00	57,00	0,06	0,19	1,70	15,00	0,08	0,17	0,03	0,03	0,01
A13Vøi	240,00	480,00	410,00	12,00	230,00	0,17	0,63	3,20	24,00	0,09	0,36	0,11	0,02	0,02
A13VDS	42,00	350,00	115,00	5,50	66,00	0,01	0,02	1,50	10,00	0,04	0,27	0,00	0,00	0,00
S15VU-1	950,00	6600,00	1600,00	120,00	850,00	7,90	3,90	26,00	410,00	2,40	2,10	0,44	0,14	0,08
S15VU-2	2200,00	6000,00	2800,00	220,00	1800,00	6,40	7,30	30,00	400,00	3,30	2,10	1,00	0,28	0,15
S15VU-3	2500,00	6800,00	2800,00	360,00	2300,00	8,90	9,00	32,00	540,00	4,60	2,70	1,30	0,35	0,20
S15Vøi-1	2800,00	5100,00	3200,00	170,00	2200,00	3,10	6,50	52,00	290,00	1,20	4,70	1,50	0,28	0,20
S15Vøi-2	2700,00	4700,00	3400,00	110,00	1900,00	2,80	5,80	45,00	200,00	0,82	2,60	2,30	0,29	0,15
S15Vøi-3	5600,00	6200,00	3700,00	200,00	4200,00	4,60	11,00	64,00	420,00	1,60	6,70	2,20	0,62	0,34
S15Sch-1	600,00	3600,00	1100,00	54,00	590,00	1,60	1,60	16,00	150,00	0,83	1,30	0,36	0,08	0,07
S15Sch-2	2200,00	7600,00	2800,00	160,00	2000,00	3,30	4,40	41,00	340,00	1,90	3,20	1,20	0,27	0,20
S15Sch-3	1600,00	5700,00	2700,00	120,00	1600,00	2,60	3,80	32,00	290,00	1,40	2,50	1,30	0,25	0,24
S15VDS-1	1400,00	6100,00	2000,00	150,00	1400,00	5,60	20,00	30,00	360,00	2,10	2,20	0,60	0,16	0,14
S15VDS-2	1800,00	6200,00	2100,00	230,00	1600,00	7,30	6,20	33,00	430,00	2,60	2,70	0,75	0,19	0,20
S15VDS-3	2400,00	5700,00	2800,00	290,00	2200,00	6,70	7,70	33,00	430,00	2,40	2,50	1,20	0,32	0,21
S15Nor	3700,00	3100,00	3300,00	370,00	19000,00	8,00	13,00	40,00	270,00	5,70	7,10	5,30	0,59	0,95
S15VU2-1	1800,00	7300,00	3200,00	180,00	1900,00	4,00	8,30	39,00	980,00	2,30	4,10	1,20	0,21	0,16
S15VU2-2	1300,00	5600,00	3400,00	220,00	1400,00	2,90	6,00	29,00	680,00	1,90	2,90	0,87	0,14	0,11
S15VU2-3	720,00	6200,00	2100,00	87,00	870,00	1,90	3,70	26,00	620,00	1,40	3,10	0,61	0,10	0,11
A15VU-1	290,00	770,00	230,00	8,90	210,00	0,32	0,72	5,20	87,00	0,11	0,73	0,09	0,03	0,02
A15VU-2	170,00	1300,00	330,00	12,00	190,00	0,55	0,59	6,90	150,00	0,16	1,60	0,06	0,03	0,01
A15Vøi-1	600,00	1200,00	590,00	27,00	520,00	0,54	1,50	6,60	67,00	0,24	1,20	0,21	0,08	0,05
A15Vøi-2	2400,00	1000,00	1800,00	68,00	2800,00	1,20	2,80	7,10	69,00	0,43	1,20	0,79	0,11	0,10
A15Vøi-3	450,00	740,00	420,00	20,00	340,00	0,37	1,10	5,10	46,00	0,17	0,83	0,18	0,07	0,04
A15Sch-1	280,00	540,00	300,00	12,00	370,00	0,23	0,51	3,60	44,00	0,14	0,66	0,15	0,05	0,04
A15Sch-2	290,00	830,00	350,00	19,00	160,00	0,36	0,70	5,30	58,00	0,20	0,87	0,16	0,04	0,04
A15Sch-3	200,00	460,00	250,00	12,00	200,00	0,20	0,46	2,90	34,00	0,13	0,44	0,11	0,03	0,02
A15VDS-1	610,00	1400,00	690,00	65,00	450,00	0,80	1,90	8,10	160,00	0,27	1,90	0,33	0,08	0,08
A15VDS-2	410,00	1000,00	460,00	24,00	310,00	0,52	1,20	6,70	120,00	0,22	1,40	0,21	0,05	0,05
A15VDS-3	560,00	720,00	320,00	17,00	770,00	0,44	1,00	5,00	83,00	0,12	0,89	0,15	0,04	0,03
A15Nor-1	580,00	870,00	800,00	74,00	2300,00	1,40	2,00	6,10	100,00	1,20	2,00	0,39	0,15	0,19
A15Nor-2	350,00	1100,00	650,00	70,00	1600,00	1,60	1,40	8,10	140,00	1,00	2,90	0,25	0,09	0,14
A15Nor-3	440,00	760,00	670,00	110,00	1900,00	1,40	1,70	6,20	95,00	1,10	1,90	0,28	0,10	0,14
A15VU2-1	250,00	2000,00	720,00	65,00	440,00	0,91	1,90	13,00	400,00	0,50	2,70	0,32	0,05	0,03
A15VU2-2	36,00	330,00	140,00	7,60	78,00	0,13	0,27	1,90	71,00	0,08	0,41	0,05	0,01	0,01
A15VU2-3	89,00	590,00	240,00	15,00	150,00	0,27	0,74	3,60	120,00	0,17	0,72	0,08	0,03	0,02

7.8 Appendix 8 – Predictors for RDA (effects and covariates)

Sample ID	Station	Season	Year	Treatment	ASPT	Al	Fe	Cd	Th	U	pH	Conduct	Temp °C	PC1	PC2
S13VU	VU	Spring	Thirteen	Ref	6,08	353,33	360,00	0,28	0,06	0,05	8,08	292	15,4	-0,6171	0,205
S13Vøi	Vøi	Spring	Thirteen	Ref	6,43	1240,00	968,33	0,37	0,15	0,07	8,27	311	15,8	-0,3489	0,5451
S13Sch	Sch	Spring	Thirteen	Ref	5,8	826,67	670,00	0,34	0,10	0,08	8,15	323	15	-0,354	0,3985
S13VDS	VDS	Spring	Thirteen	Ref	5,8	1173,33	1080,00	0,26	0,17	0,07	7,86	329	11,7	-0,2196	0,6379
A13VU	VU	Autumn	Thirteen	Ref	6,86	54,00	57,00	0,17	0,03	0,01	8,15	353	10,9	-1,3823	0,655
A13Vøi	Vøi	Autumn	Thirteen	Ref	6,35	240,00	230,00	0,36	0,02	0,02	8,28	304	9,7	-0,9421	0,319
A13VDS	VDS	Autumn	Thirteen	Ref	6,59	42,00	66,00	0,27	0,00	0,00	8,00	410	9,9	-1,5089	0,7574
S15VU	VU	Spring	Fifteen	Imp	6	1883,33	1650,00	2,30	0,26	0,14	7,97	311	8	1,2121	-1,0998
S15Vøi	Vøi	Spring	Fifteen	Ref	6,79	3700,00	2766,67	4,67	0,40	0,23	8,7	258	7,4	1,3851	-0,1606
S15Sch	Sch	Spring	Fifteen	Ref	5,88	1466,67	1396,67	2,33	0,20	0,17	7,95	274	8,6	0,8043	-0,8211
S15VDS	VDS	Spring	Fifteen	Imp	5,31	1866,67	1733,33	2,47	0,22	0,18	8,15	325	9,2	1,1938	-1,0715
S15Nor	Nor	Spring	Fifteen	Imp	4,5	3700,00	19000,00	7,10	0,59	0,95	7,88	452	6,6	2,2709	3,0829
S15VU2	VU2	Spring	Fifteen	Ref	5,4	1273,33	1390,00	3,37	0,15	0,13	8,11	258	7	1,0007	-1,6735
A15VU	VU	Autumn	Fifteen	Imp	6,43	230,00	200,00	1,17	0,03	0,02	7,89	378	7,3	-0,6998	-0,6907
A15Vøi	Vøi	Autumn	Fifteen	Ref	6,88	1150,00	1220,00	1,08	0,09	0,06	8,47	405	7,3	-0,1701	0,0211
A15Sch	Sch	Autumn	Fifteen	Ref	7	256,67	243,33	0,66	0,04	0,03	8,09	385	7,9	-0,8187	0,0887
A15VDS	VDS	Autumn	Fifteen	Imp	6,88	526,67	510,00	1,40	0,05	0,06	7,85	429	7	-0,3347	-0,489
A15Nor	Nor	Autumn	Fifteen	Imp	6	456,67	1933,33	2,27	0,11	0,16	7,88	620	7,1	0,0955	0,2204
A15VU2	VU2	Autumn	Fifteen	Ref	7	125,00	222,67	1,28	0,02	0,02	8,07	347	6,5	-0,5663	-0,9247

7.9 Appendix 9 – PCA of metals in water. Omitted from analysis as it explains less than mayfly metal concentrations.



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