Mats Willner

Macroinvertebrates as bioindicators in acid sulfate soil-affected streams:

Case study of river Perhonjoki catchment area

Mats Willner
Åbo Akademi
Miljö- och Marinbiologi
Pro Gradu- avhandling

Åbo
27.4.2020

Mats Willner

Abstract

Acid sulfate soils (a.s. soils) are responsible for deteriorating water quality and invertebrate communities in many rivers and small streams across Western Finland. Drainage of a.s. soils for both agriculture and forestry is the main reason why many Finnish streams fail to achieve good status as stated in the European Water Framework Directive (WFD). Many of the streams show signs of chronical acidity with low pH values, high metal concentrations and low species richness. Especially sensitive species from the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) are absent in acidic and highly metal-polluted streams. Individuals from the genus *Hydropsychidae* (Trichoptera) often show stress symptoms of gill-, anal papillae- and catch-net anomalies when subjected to circumstances with sublethal concentrations of metals and acidity. These responses make invertebrates good bioindicators for pollution derived from a.s. soils. Eleven streams close by or within the river Perhonjoki basin in Central-Western Finland were studied. These streams were mainly low order (1st to 3rd order) small-sized streams. The aim of this study was to identify a.s. soil impact on small streams across the river Perhonjoki catchment area, which included identification of chemical receptors and the use of bioindicators in recipient waters. The hypothesis was that (1) streams below the highest Littorina Sea level (8000 – 4000 BP) show physiochemical symptoms of a.s. soil pollution (2) which results in species composition differences between polluted and non-polluted streams and (3) the frequency of *Hydropsychidae* abnormalities is higher in polluted than non-polluted streams. Lowland (coastal) streams differed from highland (above highest sea level) streams as having higher concentrations of sulfate and aluminum, higher electric conductivity and often lower pH values. This proved that streams below the highest Littorina Sea level show symptoms of pollution derived from a.s. soils. Species composition differed significantly between a.s. soil-affected and non-affected streams, proving the hypothesis that a.s. soils alter benthic invertebrate communities. The difference in species composition between lowland and highland streams was apparent especially within Ephemeroptera species composition. Low- and highland streams did not differ in *Hydropsyche* individual gill abnormality frequencies nor *Hydropsychidae* species composition. Individuals of the genus *Hydropsyche* showed symptoms of gill abnormalities in both a.s. soil-polluted and non-polluted streams. This result was unexpected and rejected the hypothesis that gill abnormalities occur more often in a.s. soil-polluted streams.

Keywords

Hydropsychidae, abnormalities, acid sulfate soils, metal pollution, acidity, toxicology, water quality, river Perhonjoki

Mats Willner

Abstrakt

Urlakningen av syra och metaller från sura sulfatjordar (SSJ) orsakar försämrad vattenkvalitet samt problem hos vattendragens organismer i Österbotten. Torrläggningen av SSJ för jord- och skogsbruk är den största orsaken till att vattendragen i västra Finland inte uppnått god ekologisk status enligt Europeiska unionens vattenramdirektiv. Många drabbade vattendrag visar tecken på kronisk försurning med låga pH-värden, höga metallhalter och försämrad mångfald bland evertebrater. Speciellt känsliga arter från ordningarna Ephemeroptera, Plecoptera och Trichoptera saknas i svårt drabbade vattendrag. Individerna i familjen *Hydropsychidae* (Trichoptera) visar ofta symptom på gälskador, anomalier i analpapiller samt anomalier i fångstnät vid exponering till höga metallhalter och lågt pH. Effekterna av metallexponering på bottendjur gör dessa till utmärkta bioindikatorer i drabbade vattendrag. I denna avhandling undersöktes elva vattendrag både inom och i nära anslutning till Perho ås avrinningsområde i mellersta Österbotten. Största delen av vattendragen var småvattendrag (första till tredje ordningens vattendrag). Målsättningen med undersökningen var att identifiera hur SSJ påverkar vattendrag inom Perho ås avrinningsområde. Identifieringen utfördes genom att undersöka kemiska receptorer i vattendragen samt genom att tillämpa bottendjur som bioindikatorer. Hypoteserna för undersökningen var (1) att vattendragen under Littorinahavets högsta kustlinje (8 000 – 4 000 år sedan) visar symptom av påverkan från SSJ, vilket (2) syns som skillnader i bottendjurssammansättningen mellan påverkade och opåverkade vattendrag och (3) att frekvensen av gälskador hos *Hydropsyche*-nattsländor är högre hos individer i påverkade vattendrag. Resultaten av undersökningen förstärkte hypotesen att vattendrag nedanom den högsta kustlinjen är påverkade av SSJ. Halten av sulfat och aluminium var högre, konduktiviteten var högre samt minimi-pH var oftast lägre i kustnära vattendrag. Artsammansättningen i de påverkade vattendragen skilde sig signifikant från opåverkade vattendrags, vilket förstärkte hypotesen om skillnaderna i bottendjurssammansättningen. Skillnaderna i bottendjurssammansättningen var speciellt synliga inom Ephemeroptera-artsammansättningen. Det förekom inga skillnader i *Hydropsyche*-arters artsammansättning och gälskador hos *Hydropsyche*-individer framkom både i påverkade och opåverkade vattendrag. Således förkastades hypotesen om skillnaderna i frekvensen av gälskador hos påverkade och opåverkade vattendrag.

Nyckelord

Hydropsychidae, gälskador, sura sulfatjordar, metallförorening, försurning, toxikologi, Perho å, vattenkvalitet

Mats Willner

Sammanfattning

Sulfidbärande sediment samt problem med sura sulfatjordar finns runtom i världen. Största utbredningen av sura sulfatjordar i Europa finns i Finland. I Finland finns största delen av sura sulfatjordar runt Österbottens kustområde, där den intensiva landhöjningen har höjt upp järnsulfider (FeS och FeS_2), som ursprungligen sedimenterats i forna havsmiljöer. I naturliga förhållanden är sura sulfatjordar vattenmättade under grundvattennivån, men torrläggningen av sura sulfatjordar för jord- och skogsbruk sänker grundvattennivån, vilket orsakar oxidation av sedimentets järnsulfider. Oxidationen av järnsulfider sänker markens pH-värden och mängder med skadliga metaller frigörs från markens mineraler. Urlakningen av syra och metaller från sura sulfatjordar orsakar försämrad vattenkvalitet samt problem hos vattendragens organismer. Urlakningen av syra och metaller är största orsaken till att vattendragen i västra Finland inte uppnått god ekologisk status enligt Europeiska unionens vattenramdirektiv. Många drabbade vattendrag visar tecken på kronisk försurning, med låga pH-värden, höga metallhalter och försämrad mångfald bland evertebrater. Speciellt känsliga arter från ordningarna Ephemeroptera, Plecoptera och Trichoptera saknas i svårt drabbade vattendrag. Individerna i familjen *Hydropsychidae* (Trichoptera) visar ofta symptom på gälskador, anomalier i analpapiller samt anomalier i fångstnät vid exponering till höga metallhalter och lågt pH. Effekterna av metallexponering på bottendjur gör dessa till utmärkta bioindikatorer i drabbade vattendrag.

I denna avhandling undersöktes elva vattendrag både inom och i nära anslutning till Perho ås avrinningsområde i mellersta Österbotten. Vattendragen som undersöktes inom Perho ås avrinningsområde är Åivobäcken, Hömossadiket, Kainobäcken, Tastulanoja, Saarivedenoja, Lammasoja, Kaihianoja, Kivioja, Puro och Penninkijoki. Korplaxbäcken (finska Korpilahdenoja) rinner bredvid Perho å, men dess vatten mynnar rakt ut till Bottenviken norr om Perho å. Korplaxbäcken är en av de största bäckarna i området, vilket är orsaken till att bäcken blev vald med i undersökningen. Vattendragen i undersökningen är i huvudsak småvattendrag med mindre än 100 km^2 stora avrinningsområden. Penninkijoki skilde sig från andra vattendrag genom att dess avrinningsområde är större än 100 km^2. Penninkijoki blev vald med i undersökningen på grund av dess stora orörda myrområden utför bra referens för områden med liten mänsklig påverkan.

Målsättningen med undersökningen var att identifiera hur sura sulfatjordar påverkar vattendrag inom Perho ås avrinningsområde och samtidigt undersöka om *Hydropsyche*-nattsländelarver är lämpliga bioindikatorer för påverkan av sura sulfatjordar i småvattendrag runt Perho ås avrinningsområde. Hypoteserna för undersökningen var (1) att vattendragen under Littorinahavets högsta kustlinje (8 000 – 4 000 år sedan) visar symptom av påverkan från sura sulfatjordar, vilket (2) syns som skillnader i bottendjurssammansättningen mellan påverkade och opåverkade vattendrag och (3) att frekvensen av gälskador hos *Hydropsyche*-nattsländor är högre hos individer i påverkade vattendrag.

Identifieringen av påverkan från sura sulfatjordar utfördes genom att undersöka kemiska receptorer i vattendragen samt genom att tillämpa bottendjur som bioindikatorer. Vattenprovtagning utfördes på hösten 2018 och våren 2019. Från proven analyserades pH, konduktivitet, aciditet, halten metaller, halten sulfat och totala halten organiskt kol. Bottendjursprovtagningen utfördes hösten 2018 med sparkhåvsmetoden. Provtagningen utfördes genom att kombinera tre parallella 30 sekunders prov från varje provplats. I mån av möjlighet bestämdes arterna ned till art- eller släktnivå, men vidare jämförelser av totala artsammansättningar mellan provplatserna utfördes på familjenivå.

Resultaten av undersökningen förstärkte hypotesen att vattendrag nedanom den högsta kustlinjen är påverkade av sura sulfatjordar. Konduktiviteten samt halten av sulfat och aluminium korrelerade signifikant med höjden över havet. Konduktiviteten samt halten sulfat och aluminium var signifikant högre i kustnära vattendrag. Minimi-pH var oftast lägre i kustnära vattendrag, vilket bevisades med en svag men signifikant korrelationen mellan pH och höjden över havet. Artsammansättningen i de påverkade vattendragen skilde sig signifikant från opåverkade vattendrags, vilket förstärkte hypotesen om skillnaderna i bottendjurssammansättningen. Skillnaderna i bottendjurssammansättningen var speciellt synliga inom Ephemeroptera-artsammansättningen. Sura sulfatjordspåverkade vattendrag innehöll flera individer från familjen *Beatidae*. Opåverkade vattendrag hade däremot mindre individer från familjen *Beatidae* och flera individer från familjen *Leptophlebiidae*. Det förekom inga skillnader i *Hydropsyche*-arters artsammansättning och gälskador hos *Hydropsyche*-individer framkom både i påverkade och opåverkade vattendrag. Således förkastades hypotesen om skillnaderna i frekvensen av gälskador hos påverkade och opåverkade vattendrag.

Hydropsyche-arter kan anses som bra bioindikatorer i småvattendragen inom avrinningsområdet för Perho å. Arterna är känsliga för metaller och sura förhållanden, vilka är typiska för sura sulfatjordspåverkade vattendrag i nedre delen av Perho å. *Hydropsyche*-arter saknades i många opåverkade och påverkade vattendrag, vilket försvårade jämförelsen av vattendragen. Det vore intressant att utföra exponeringsexperiment med *Hydropsyche*-arter i vattendragen där arten saknades i undersökningen. Exponeringsexperimenten skulle ge oss mera information om faktorer som påverkar *Hydropsyche*-arters utbredning och känslighet för metaller.

Problemen från sura sulfatjordar, med höga metallhalter samt låga pH-värden, korrelerar normalt med högt vattenflöde i vattendragen. Hela år 2018 och våren 2019 var extremt torra i området, vilket orsakade torka och lågvattenflöde i många av de undersökta vattendragen. Bristen av högvattenflöde i de undersökta vattendragen ledde till situationer där metallhalter och pH-värden var bättre än normalt i vattendrag påverkade av sura sulfatjordar. Dessa onormala förhållanden minskade skillnader mellan påverkade och opåverkade vattendrag. Förhållanden under år 2018 och våren 2019 har varit optimala för oxidationen av sedimentets sulfider. Därmed är sannolikheten mycket stor för en metall- och surhetschock under nästa högvattenflöde. Det är viktigt att repetera undersökningen efter följande surhetschock. Resultatet från denna undersökning fungerar som en bra referens för förhållanden före den förväntade surhetschocken.

Contents

1 Introduction 1
2 Background 3
2.1.1. The formation of sulfide-bearing sediments 3
2.1.2. Sulfide-bearing sediments in Finland 4
2.1.3. From sulfide sediment to acid sulfate soil 4
2.1.4. Acid sulfate soils in Finland 5
2.2.1. Perhonjoki river in C-W Finland 8
2.2.2. Water quality in river Perhonjoki 8
2.3.1. Invertebrates as bioindicators 9
2.3.2. *Hydropsychidae* ecology 10
3.3.3 *Hydropsychidae* as bioindicator for metal pollution 12
3 Materials and methods 13
3.1 Study area 13
3.2 Water quality data 15
3.3 Invertebrate sampling 15
3.4 Trichoptera *Hydropsychidae* analyses 16
3.5 Statistical analyses 17
4 Results 18
4.1 Water quality 18
4.2 Species composition and *Hydropsyche* abnormalities 19
5. Discussion 25
5.1 Water quality 25
5.2 Effects on invertebrate communities 28
5.3 *Hydropsyche* gill abnormalities 32
6 Conclusion 33
Acknowledgements 34
References 35

1 Introduction

Acid sulphate soils (a.s. soils), largely found around coastal areas in western Finland, are highly responsible for deteriorating water quality in many Ostrobothnian streams. Oxidation of iron sulfides (FeS and FeS_2) through human activities (for example drainage of forest- and farmland) has caused raised metal concentrations, raised electric conductivity (EC), raised sulfate concentrations and lowered pH values in recipient streams (e.g. Åström & Åström 1997; Åström & Björklund 1995). Deteriorating water quality has led to poor hydro-chemical conditions for in-stream biota. A combination of high metal concentrations and low pH values has caused problems for many sensitive species. Many Ostrobothnian rivers suffered from severe acidity between the years 2006 and 2007. This was due to a warm and dry summer in 2006, which was followed by heavy rainfall in the autumn. Severe acidity led to large fish deaths in many rivers. The extent of the events was so huge that it caught the attention of mainstream media and showed the urge for new practices within water and acid sulfate soil management.

In Finland, leaching of metals from acid sulfate soils is calculated to be a larger source of metal pollution to water courses than that of industries combined (e.g. Sundström et al. 2002). The extent of metal loads and acidity from a.s. soils is the main reason to why streams in western Finland have not reached the goals stated in the European Water Framework Directive (WFD). Some of the affected rivers and lakes are well documented and there are many studies that describe the fluctuation in their water quality (e.g. Toivonen & Österholm 2011). However, it is problematic to assess the status of streams solely by monitoring physiochemical water quality data. Impacts of contaminants are better shown by combining physiochemical water quality monitoring with toxicological tests and observations on the in-stream biota.

High concentrations of iron (Fe) and aluminum (Al) combined with low pH values have proved to be lethal for many stream organisms. High concentrations of Fe combined with low pH (5–6) have proved to cause damage to brown trout *(Salmo trutta*) gills and heighten blood glucose values (Peuranen et al. 1994). Lestijoki river water, a river north of river Perhonjoki in Central Ostrobothnia, has shown to have a negative impact on *S. trutta* osmoregulatory ability at their smolt-stage, lowering the fitness of individual trout (Soivio et al. 1998). Malte and Weber (1988) showed that rainbow trout (*Oncorhynchus mykiss*) suffered from severe respiratory stress and died subjected to artificially created soft water with low pH value (5.0) and high Al concentration. These values are typically found in Ostrobothnian streams, which suggests that sensitive salmonid species are absent in many rivers. Vuorinen et al. (1993) studied the combined effects of Al and pH values on pike (*Esox Lucius*), whitefish (*Coregonus lavaretus pallasi*), pike perch (*Stizostedion luciperca*) and roach (*Rutilus rutilus*). In their studies, they noticed that the higher the Al concentrations were, the more toxic the water was at every pH value (ranging from 4 to 6). Their studies showed a direct correlation between Al concentration and mortality. Their results indicated that pike is the most tolerant species and roach seems to be the most sensitive out of the four species in their study. Their results indicate that some of the rivers in Ostrobothnia, which suffer from chronic acidity, might be completely empty of fish. However, compared to highly mobile fish species, the less mobile stream invertebrates are even more sensitive to changing water qualities. That is why they are perfect bioindicators and indicate differences in water quality not only between streams, but also between different parts of a stream. Especially the abundance or absence of different species of the caddisfly *Hydropsyche* is known to be good bioindicators for stream water quality (e.g. Tolkamp & Highler 1983; Basaguren & Orive 1990; Vuori & Parkko 1996). Mayflies from the *Heptageniidae* and *Ephmerellidae* orders are also shown to be sensitive to deteriorating water quality (e.g. Rainbow et al. 2012; Awrahman et al. 2016). Invertebrates are important for benthic ecosystems. They maintain ecosystem energy flows by converting organic material to prey items for larger consumers (Covich et al. 1999). Absence of important key species can change energy flows, which can have devastating effects on the whole ecosystem. This makes studies on invertebrate communities in polluted streams important.

Many studies focus on larger rivers and less is known about the smaller streams around the Perhonjoki river catchment area, where the fluctuation in water quality is even larger than in recipient rivers. Especially sensitive are the smaller streams whose entire catchment area is situated below the highest Littorina Sea level (HSL). Many of these streams lack either water quality data, biological data or even both.

The aim of this thesis was to (1) gain more knowledge about water chemistry from the smaller streams around the river Perhonjoki catchment area, (2) identify the extent of a.s. soil impact on these streams, (3) obtain data of species composition especially with emphasis on the composition of EPT-species (Ephemeroptera, Plecoptera & Trichoptera-species) and (4) identify the suitability to use *Hydropsychidae* (Trichoptera) larvae as bioindicators in these small streams.

The hypothesis for this study was (1) streams below HSL (approximately 100 m above sea level to date) suffer from pollution, derived from drainage of a.s. soils. This should be noticeable from high EC values, high metal concentrations, low pH-values and high sulfur concentrations in lowland (below HSL) streams. The difference in water quality between lowland and highland (above HSL) streams should result in (2) differences in species composition. Sensitive species from the orders Ephemeroptera and Trichoptera should be more abundant in non-polluted streams compared to polluted streams. Lowland streams should also show (3) a higher frequency of tracheal gill abnormalities on *Hydropsychidae* larvae compared to streams higher up in the system.

2 Background

2.1.1. The formation of sulfide-bearing sediments

Rickard (2012) comprehensively describes the formation of sulfide-bearing soil sediments, which are formed under anaerobic conditions, where sulfate-reducing microbes (SRM) metabolize organic material from sea water in the presence of iron-containing minerals. Sulfate is an easily soluble salt that commonly occurs in seawater. Sulfate-reducing microbes use sulfur (S) from sulfate (SO_4) instead of oxygen (O) as their electron acceptor in their metabolism. For example, lactate is oxidized in SRM by the following reaction:

$$2\ CH_3CHOHCOO^- + 3\ SO4^{2-} = 6\ HCO_3^- + 3\ HS^- + H^+$$

The reduction of sulfate to sulfide, through SRM activity, can be expressed by the reaction:

$$SO_4^{2-} + 10\ H^+ + 8\ e- = H_2S + 4\ H_2O.$$

As shown above, the metabolic activity of anaerobic sulfur-reducing microbes makes the formation of sulfides possible in marine environments. The produced sulfides (H_2S or HS^-) react with iron to produce precipitates of iron sulfides. Metastable FeS is commonly Mackinawite (FeS_m), which is a widespread mineral in low-temperature aqueous environments. FeS does not precipitate easily in average seawater nanomolar total dissolved Fe concentrations. Precipitants are usually found in environments where the total concentration of dissolved Fe(II) is higher than normal. The formation of FeS_m can be described with the following simplified reaction between Fe(II) and S(-II):

$$Fe^{2+} + H_2S \rightarrow FeS_m + 2H^+$$

This reaction is also influenced by iron-reducing prokaryotes in the sediment column, which resemble SRM in their microbial activity, but use Fe(III) as their electron acceptor. The most common sulfide mineral on the earth's surface is the stable mineral Pyrite (FeS_2), which is also known as fool's gold. The solubility of the cubical pyrite mineral is very low in neutral pH conditions. Precipitation of pyrite is a complex series of possible pathways, due to which precipitation of pyrite minerals can occur. Pyrite can precipitate through e.g. these reactions with FeS_m:

Reaction 1

$$FeS_m + H^+ \rightarrow Fe\ (II) + HS^-\ \text{(step 1)}$$

$$Fe(II) + S_2(-II) \rightarrow FeS_2\ \text{(step 2)}$$

$$FeS_m + H^+ + S_2(-II) \rightarrow FeS_2 + HS^-$$

Reaction 2

$$FeS + H_2S \rightarrow FeS_2 + H_2$$

In reaction 1, the metastable iron sulfide fully dissolves to Fe(II) and HS^-, when reacting with H^+. The formed iron-ion react with the sulfide ion to produce pyrite and sulfide. In reaction 2, the metastable iron sulfide reacts with hydrogen sulfide to produce pyrite and hydrogen gas. Both reactions eventually give rise to pyrite iron sulfide (FeS_2)

2.1.2. Sulfide-bearing sediments in Finland

The formation of sulfide sediments was intensive at the Littorina Baltic sea phase (8000–4000 BP). In that period, the sea went through a phase of post-glacial relatively organic-rich brackish water. This is seen from black iron sulfide deposits around the Finnish coast. Sulfide-bearing sediments in Central-Western Finland are composed of silts, clay and sand, with silts being the most common sediment size (e.g. Åström & Björklund 1997). Clay sediments are composed of the minerals of weathering rocks in the region. Deng et al. (1998) showed that phyllosilicates from acid sulfate soils around Petalax in Ostrobothnia consisted mainly of micas, kaolinites, chlorites and vermiculites. K-feldspars, quarts and amphiboles were also present in the samples. The majority of metals increase in concentration, when moving from coarser material (sand and silt) to clay. Many toxic metals (e.g. Al) are bound in their mineral structures in various types of phyllosilicates. Åström and Björklund (1997) also showed that Al, Fe, K, Mg and Zn are the most abundant metals associated with acid sulfate soils. The concentration of metals was twice as high in their samples compared to fine-fraction non-acid sulfate soil sediments in the same area.

2.1.3. From sulfide sediment to acid sulfate soil

Iron sulfide sediments are neutral in their natural waterlogged state, but they do possess the ability to become acid, because of their sulfide concentration. These unoxidized sediments are usually called potential acid sulfate soils. Pyrite (FeS_2) is a stable molecule under reduced conditions submerged below the groundwater table. Human activities, such as drainage of farmlands and peatland forests, lowers the groundwater table, which enables atmospheric oxygen to react with sulfidic sediments. This reaction starts a complex weathering process. Weathering of pyrite is slow in pH neutral conditions, and it takes some time for the process to accelerate. The process is often catalyzed by acidophilic bacteria, which Wu et al. (2013) found in Risöfladan a.s. soil area close to Vaasa, suggesting that the temperature is high enough for acidophilic bacterial growth in Ostrobothnian soils during summer. The oxidation of pyrite and monosulfides leads, finally, to the formation of iron hydroxides ($Fe(OH)_3$) and sulfuric acid (H_2SO_4). Simplified oxidation reaction for hydrolysis of iron, monosulfides and pyrite are (van Breemen 1973 in Österholm 2005):

$$Fe^{3+} + 3\,H_2O \rightarrow Fe(OH)_3 + 3\,H^+ \text{ (at low pH)}$$

$$4\,FeS + 9\,O_2 + 10\,H_2O \rightarrow 4\,Fe(OH)_3 + 4\,H_2SO_4$$

$$4\,FeS_2 + 15\,O_2 + 14\,H_2O \rightarrow 4\,Fe(OH)_3 + 8\,H_2SO_4$$

The formation of these severe acids lowers the pH value in poorly buffered oxidized soil horizons. pH values drop from neutral (7–8) to less than 5 in the oxidized zone and rise back to neutral in the unoxidized parent material. Low pH values have been measured in a.s. soils all around Central-Western Finland (e.g. Wu et al. 2013; Åström & Björklund 1997; Åström 1998; Palko & Yli-Halla 1990; Joukainen & Yli-Halla 2003). Acidification of the soil increases the solubility of metals such as Al, Cu and Zn from soil minerals (e.g. Palko & Yli-Halla 1990). The produced cocktail of acidity and soluble metals is washed out with drainage water and effect water quality in recipient streams. Acid sulfate soil drainage water has been shown to have high concentration of sulfate, low pH, high electric conductivity and high concentrations of metals (e.g. Bärlund et al. 2005). Nystrand et al. (2012) showed that there is a difference in metal composition between low order a.s. soil-affected streams and non-affected streams in South-Western Finland. Metals, such as aluminum were mainly found dissolved in stream water, whereas non-affected rivers and streams had aluminum bound in particle or colloidal form, which were derived from erosion of clay soils. Even if metal concentrations were high in non-affected streams, the metals were in particle form, which are less bioavailable and harmful for stream biota. The fact that metals are mainly found in dissolved form in a.s. soil-impacted streams together with low pH values is what makes a.s. soil drainage extremely toxic.

2.1.4. Acid sulfate soils in Finland

The largest areas of acid sulfate soils in Europe are found in Finland. Palko (1994) estimated that there are around 336 000 ha of acid sulfate soils in Finland, of which most of them are found around the Gulf of Bothnia. Some estimates only calculate the extent of acid sulfate soils on agricultural land and uses different international criteria of soil taxonomy (e.g. Yli-Halla et al. 1999). The total area of acid sulfate soils in Finland is much greater than those estimates, which was also suggested by Beucher et al. (2015). Their studies showed that the previous estimates of acid sulfate soil occurrence were wrong, and a more detailed mapping was necessary. New knowledge about acid sulfate soil sediments has changed our understanding of the problems occurring in different sediment types. Traditionally, acid sulfate soils have been categorized as fine-grained sediments of sulfide-bearing silt and clay, but recent studies show that also coarse sand sediments, with bad buffering capacities, might be potential acid sulfate soils (e.g. Mattbäck et al. 2017).

Åström and Björklund (1997) analyzed 317 samples from the Quark area in Ostrobothnia and found that the median sulfur concentration was 0.54% and the maximum concentration found was 1.78%. However, they pointed out that some of the sites were heavily oxidized, which means that some of the sulfur might have leached from the sediment, lowering the concentration of sulfur in their study. Åström (1998) showed that the sulfur concentration greatly changed between the oxidized and reduced zone. The oxidized zones had sulfur concentrations between 0.04% and 0.46%, whereas the transition zones (between oxidized and reduced zone) had sulfur concentrations between 0.6% and 2.1%. Even if the sulfur concentration in Ostrobothnian acid sulfate soils is quite low compared to tropical areas, they have the potential of causing serious acidity, due to poorly buffered soils in the region.

Due to rapid post-glacier land uplift (approximately 8 mm/year) in Ostrobothnia, Central-Western Finland, these sulfide-bearing sediments can be found far away inland, below the highest Littorina Sea level (approximately 100 m above sea level to date). Due to flat topography around the Gulf of Bothnia, these sediments have been covered with peat, which has naturally raised the ground water level and prevented sulfide oxidation. These sulfide-bearing sediments are naturally neutral (pH around 7), when left in their natural waterlogged state. Extensive land use after the Second World War started the massive acidification of streams in Central-Western Finland (Åström et al. 2005). It began with intensive drainage of peatland for forestry in the 1950s, when ditches where dug through the peat layer down to the mineral soils, exposing sulfide-bearing sediments for atmospheric oxygen. The soil profile from a drained peat-covered forest in Central-Western Finland has typically a thin peat layer, followed by an oxidized (grey) layer of acid sulfate soil, ending in the unoxidized dark monosulfide-bearing sediment (Fig. 1). The drainage of forest was most intense between the 1950s and the 1970s and only small new areas are drained at present, but maintenance ditching is common in the area even to date.

Fig. 1. A traditional soil profile from a peat-covered acid sulfate soil in C-W Finland. The ditch has been dug in a thin peat layer (approximately 0.5 m), followed by an oxidized a.s. soil layer (grey). New maintenance ditching has revealed unoxidized sulfide-bearing parent sediment (black) underneath the oxidized layer. Picture from Kiimakorpi at Hömossadiket catchment area, Nedervetil, Finland.

Farmlands were traditionally drained by open ditches in small patches. These patches were badly suitable for large modern equipment, which led to the use of subsurface drainage pipes. Installation of subsurface drainage pipes started to intensify in the 1960s and many formerly open ditch farmlands were transformed to subsurface drainage. This trend has continued to the present day. Subsurface drainage pipes are more effective at draining farmlands compared to the traditional open ditch system and the effects of drainage pipes have shown to exceed down to depths of 2 m (Joukainen & Yli-Halla 2003). Lowering of the ground water table, due to both open ditch and subsurface drainage, has enabled atmospheric oxygen to penetrate down to deeper sulfidic layers, starting the oxidization process of sulfides in the sediments. Österholm and Åström (2002) showed that Rintala area in Southern Ostrobothnia had agricultural acid sulfate soils with distinctive zones in soil profiles. The uppermost zone was the plough layer (depth 0–35 cm), followed by the acidic zone (depth 35–140 cm), which was followed by the weakly acidic transition zone (depth 140–170 cm) and ending in the parent material (depth > 170 cm). The soil profile was divided into different zones according to changes in pH values. The pH value dropped from > 4.5 in the limed plough layer to < 4.5 and rose again slowly in the transition zone. The pH value rose again to above 6 in the parent sediment. Using old redox potential and pH measurement data Virtanen et al. (2017) showed that also soil type affect oxidation depth.

Coarser silt and sandy soils enable atmospheric oxygen to penetrate more easily to deeper depths compared to finer clay soils.

2.2.1. Perhonjoki river in C-W Finland

The river Perhonjoki has its sources in the highlands of Suomenselkä area between the regions of Central Ostrobothnia and Central Finland, in the municipalities of Perho, Kyyjärvi and Kivijärvi, where it collects its waters from small headwater lakes. These lakes are situated approximately 200 m above sea level. The river has a catchment area of 2524 km^2 and a length of 160 km from headwater lakes out to the Baltic Sea a few km north of the town of Kokkola. The topography is flat, which is typical for Ostrobothnia. The flat river valleys are covered with layers of peat and the waters are normally dark colored and slightly acidic from humic acids.

2.2.2. Water quality in river Perhonjoki

Water quality in the lower parts of the river shows seasonal and annual variations. Water acidity and metal concentrations fluctuate heavily. The cause of these fluctuations in the lower parts of the river (especially below 40 m above sea level) is heavy land use on areas with acid sulfate soils. As a result, sulfur concentrations are low in the upper river system around the municipalities of Kaustinen and Veteli, whereafter they rise towards the estuary. Periods of severe acidity has been a problem in the lower parts of the Perhonjoki river. The years 2006 and 2007 were especially problematic. A warm dry summer, followed by heavy rainfall, led to a sudden drop in pH values in the autumn of 2006. pH values fell below 5.0 and the lowest measured pH value was 4.4 in the Perhonjoki river. At the same time, the pH value dropped in nearby Såkabäcken (a 105 km^2 large stream in the lower part of the Perhonjoki river catchment area) to even as low as below 4.0 for three months in a row. This severe period of acidity led to a widespread fish death and it took several years for the river to recover. Similar drops in pH values for lower parts of the river have been measured in the years 1974, 1976, 1996 and 1997, when pH values fell below 5.0 (e.g. Airiola et al. 2016).

Acid sulfate soils divide the river into two distinctive halves. Vuori monitored the water quality of the lower and upper parts of the river in 1997. The average pH value for the lower part of the river was 6.0 (n = 8) and the minimum measured pH value was 4.8. The pH value dropped even lower a bit upstream below a well-known acid stream called Hömossadiket. The measured pH value was as low as 4.5. The average pH value higher up in Karjalankoski (Veteli) was 6.4 (n = 8) with a lowest pH value of 6.1 (Fig. 2). Aluminum concentration was on average 358 µg/l (n = 8) in Karjalankoski and 837 µg/l in the lower part of the river. Maximum aluminum concentration was 1980 µg/l close to the estuary and even higher below Hömossadiket

(4190 µg/l). The highest measured aluminum concentration was only 470 µg/l at Karjalankoski. Similar differences between the upper and lower parts of the river can be seen also with other metal concentrations (Vuori 2002).

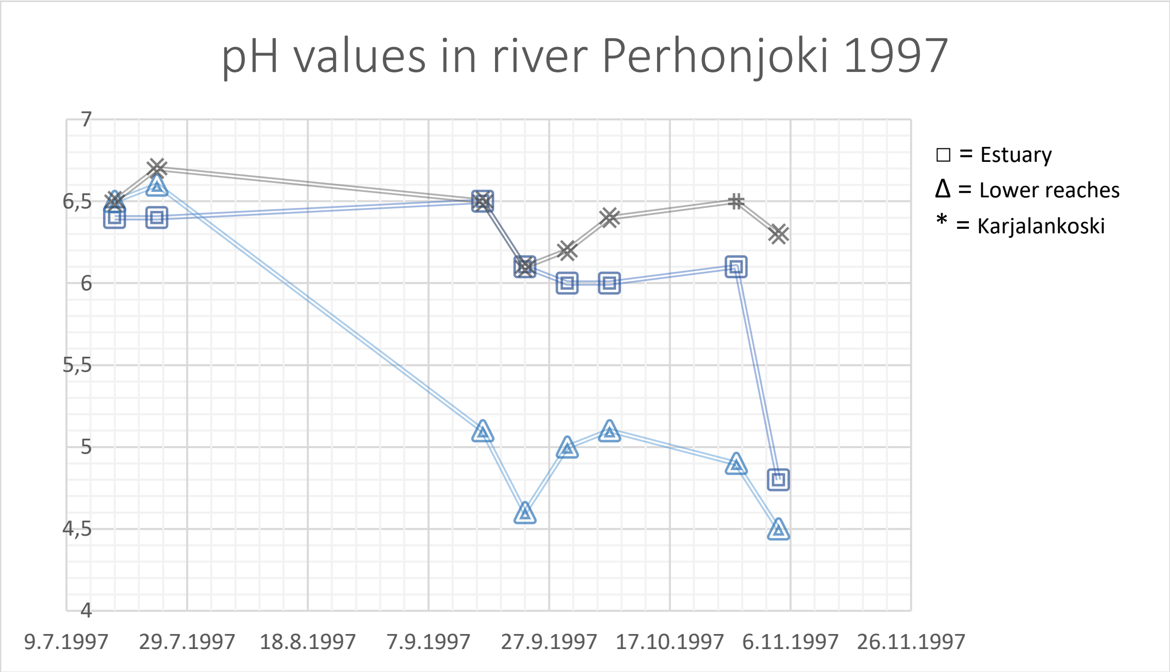

Fig. 2. pH values at three sites in the Perhonjoki river from July to November 1997. The sites are estuary (below Hongabäcken), lower reaches in Kokkola (below Hömossadiket) and Karjalankoski (Veteli). pH values (y-axis) dropped at the estuary and the lower parts of the river to below 5.0, whereas Karjalankoski remained above 6.0 through the entire monitoring period. Graph made from data obtained by Vuori (2002).

Data from Vuori (2002) show a distinctive pattern regarding water quality in the Perhonjoki river. pH values fluctuate more in the lower part of the river compared with Karjalankoski, where the fluctuation in 1997 was minimal. Acid streams affect the water quality by lowering pH values and raising metal concentrations in the river. Low pH values and high metal concentrations occurred in autumn, when heavy rainfall raised water levels, flushing acid water to the river from smaller streams. The effect of smaller streams on the Perhonjoki river water quality is enormous, which make studies of these small streams important. Better knowledge of these acidic small streams will give us more knowledge about the hazards that affect the lower parts of the river.

2.3.1. Invertebrates as bioindicators

Invertebrates are considered excellent bioindicators because of their sedentary life, which makes them vulnerable to changes in water quality through their lifecycle. They dwell in the bottom of streams and seldom move for a long distance. However, some species have been reported to drift downstream when the water quality has changed for the worse (e.g. Ormerod et al. 1987; Raddum & Fjellheim 1987). McLay (1968) studied streams at the South Island of New Zealand. Results from his studies showed that drifting of invertebrates peaked usually after floods and at nighttime. This means that invertebrates can colonize and disperse to a new area close by their original site.

Invertebrates are often used to assess impact of metals on in-stream biota in laboratory experiments, *in situ* toxicity experiments and field collections from locations using standardized methods of invertebrate sampling. Laboratory experiments usually consist of aquariums with manipulated stream water (e.g. Awrahman et al. 2015; Ruuth 2017). *In situ* toxicity tests involve transportation of target species from unpolluted streams to polluted streams, where individuals are usually put in constructed cages and anchored to the bottom substrate. They are then left exposed to the polluted stream water for a short time period. Examples of *in situ* toxicity tests are found in e.g. Lepori & Ormerod (2005) and Kowalik & Ormerod (2006). Field collection is usually carried out with a semi-quantitative kick-net method at stream rifles. This method is commonly used in e.g. assessing stream ecological status in the European Water Framework Directive. More information on the methodology for using kick-net sampling can be found in e.g. Heino et al. (2002) and Mykrä et al. (2006). Qualitative invertebrate sampling has also been used in some instances to collect invertebrates from streams (e.g. Lenat 1988). In both cases the caught individuals are counted, and the species composition gives a good indication of the stream quality at the target location. Other field collection experiments are focused on one or few specific target species, where collected individuals are for instance analyzed for their bioaccumulated metals (e.g. Awrahman et al. 2016). The targeted species are often chosen from the orders Ephemeroptera, Plecoptera or Trichoptera. These so-called EPT-species are good bioindicators and their abundancy and composition are often used in assessing e.g. the impact of land use on stream ecology (e.g. dos Reis Oliveira et al. 2019 article in press).

2.3.2. *Hydropsychidae* ecology

Caddisflies from the family *Hydropsychidae* live their larval stage in the stony bottom of rivers and streams, where many of them build a silken net to filter food particles from the water surface. Because of this net-spinning habit, they are also called net-spinners (Badcock 1976). They ingest fine particulate organic matter (FPOM) as their primary food, which they filter from the water. FPOM is a broad variety of different food sources that consist e.g. of algae, particles derived from breakdown of larger particles (e.g. leaf and branches), feces, bacteria etc. (e.g. Allan & Castillo 2007; Hutchens Jr. et al. 2017).

Hydropsyche larvae go through five instar stages, before they go through a pupae stage and emerge as adults. Andersen & Klubnes (1983) studied the life history of two *Hydropsyche* larvae (*Hydropsyche siltalai* and

Hydropsyche pellucidula) in a West Norwegian river. Their results indicated that both species completed their life cycle within a year. However, the species differed in both timing of the pupae stage and their instar stage under the winter months. *Hydropsyche siltalai* went through the winter as fourth instar larvae, whereas *H. pellucidula* went through the winter months as fifth instar larvae. *Hydropsyche pellucidula* emerged as pupae from May to end of July, whereas *H. siltalai* emerged as pupae in July and August. Their findings indicate that *H. pellucidula* individuals are larger in autumn and winter months compared to *H. siltalai* individuals. Similar results with differences in larvae sizes between overwintering *Hydropsychidae* species has been reported in e.g. Hildrew and Edington (1979).

Hydropsychidae species are nearly identical in their feeding habit and lifecycle, indicating heavy competition between species. Even though their niche seem identical, different species have been observed coexisting in streams. Malas and Wallace (1977) showed that different species of net-spinning Trichopteran larvae coexisted in different parts within a stream. Some species are more abundant on the upper side of stones, whereas some thrive underneath the stones. Their results also indicated that species differed in their stream water velocity preferences. Hildrew and Edington (1979) showed that *H. pellucidula* and *H. siltalai* coexist in the river Usk in Wales. However, there was a significant difference in current velocity preferences between species. *Hydropsyche siltalai* was mainly found at faster flowing rapids, whereas *H. pellucidula* was also found in pools with calmer water.

Hildrew and Edington (1979) also showed that *H. pellucidula* and *H. siltalai* diets consisted of higher plant material, filamentous algae and bryophyte fragments. However, *H. pellucidula* differed from *H. siltalai* by having also ingested larger animal parts. Hildrew and Edington suggested that this difference in dietary composition was due to size difference between these two species in autumn months. Larger individuals construct larger nets, which are capable of catching larger food particles. Wallace (1975) reported that there was a difference in capture-net mesh opening size and ingested food-particle type and size between three North American *Hydropsychidae* species. Food particle size correlated with the sizes of capture-net mesh sizes.

These small but significant differences between species make it possible for different species to coexist. However, these small differences can give rise to different species thriving at different locations, which can result in a zonation of *Hydropsychidae* species within a stream system. It has been shown that different stretches of small streams and larger rivers have their own characteristic species. Different species thrive in different locations depending on stream width, velocity and bottom substrate. For instance, *H. saxonica* thrives in smaller faster running waters of the first-order and *H. siltalai* is predominantly found in larger streams and rivers in areas with hilly topography. *Hydropsyche pellucidula* is found in many types of streams and rivers. *Hydropsyche pellucidula* often occur together with other *Hydrospyche* species, such as *H. siltalai*, but *H. pellucidula* is often found further downstream, because of its capability to tolerate slower current velocities. *Hydropsyche angustipennis* is often found in different sized lowland streams. *Hydropsyche angustipennis* is resilient to low oxygen values and pollution, and is sometimes found also in stagnant water. *Hydropsyche angustipennis* is sometimes found together with other *Hydropsyche* species, such as *H. pellucidula* and *H. siltalai*. However, *H. angustipennis* is a more typical lowland species and is seldom found in streams higher up in the river system (e.g. Tolkamp & Higler 1983; Basaguren & Orive 1990; Badcock 1976).

3.3.3 *Hydropsychidae* as bioindicator for metal pollution

Trichopteran caddisfly larvae of the taxonomic family *Hydropsychidae* can be found in all types of running waters. *Hydropsychidae* larvae have tracheal gills on their abdomen, which eases the identification of these species. Easily visible gills make *Hydropsychidae* caddisflies interesting species for biomonitoring acid and aluminum rich waters. *Hydropsyche* larvae have been used to assess pollution levels in metal polluted streams around the world. Poor water quality has shown to induce abnormalities in larval tracheal gills (e.g. Vuori & Parkko 1996), abnormal anal papillae (e.g. Vuori 1996) and abnormalities in larval constructed nets (e.g. Vuori 2002). Laboratory studies (e.g. Awrahman et al. 2015) and field studies (e.g. Rainbow et al. 2012) conducted on *Hydropsyche* species show accumulation of metals in larval tissues, which cause stress and abnormalities in sub-lethal concentrations (e.g. Ruuth 2017; Vuori & Kukkonen 1996). *Hydropsyche* tissue metal concentrations have shown to correlate negatively with Ephemeroptera species abundancies (e.g. Rainbow et al. 2012; Awrahman et al. 2016). Vuori (1993) showed that Al and Fe concentration in *Hydropsyche* larval tissue correlated with stream metal concentrations. Vuori and Kukkonen (1996) showed that the Al concentration was significantly higher in individuals with gill abnormalities compared to healthy individuals.

Accumulation of metals on and in *Hydropsychidae* larvae follows various ways. Vuori (1993) reported that iron flakes coated aquatic moss and larvae in acidic streams. Vuori and Kukkonen (1996) showed that Fe accumulated on larvae, but molting led to lowered larval Fe concentrations. Lower larval Fe concentrations after molting proved that most of the Fe accumulated on the larval surface. Vuori (1996) pointed out that Al might have caused darkening of anal papillae through an increase in accumulation of Al^{3+}-ions in anal papillae epithelial cells and that Al usually form milky aluminum hydroxides on gill tuffs, which was also noticeable in his study. Vuori (1993) showed that larval Fe and Al concentrations correlated positively with water metal concentration. The same correlation was not observed for other metals such as Zn, Cu, Pb, and Cd. This difference could be achieved by different ways of accumulation. Differences in accumulation between Fe and Al compared to other metals could be caused by the fact that Fe and Al react with tissues on the larval surface. Other metals could accumulate e.g. through ingestion. Ingestion of metal-contaminated food have shown to raise *Hydropsyche* larval metal concentrations. Awrahman et al. (2015) showed that *Hydropsyche* larval As and Ag concentrations rose when ingesting labeled As and Ag food particles. Vuori and Kukkonen (1996) also reported that levels of Cd and Cu concentrations were in fact higher after molting than before molting. This means that Cd and Cu are most likely bound to tissues in the larvae after ingestion of metal particles.

3 Materials and methods

3.1 Study area

Fifteen locations in eleven different streams were studied in this thesis (Table 1). Streams were scattered across or close by the river Perhonjoki catchment area in Central Ostrobothnia, Finland (Fig. 3). All except one stream were located within the river Perhonjoki catchment area. Korpilahdenoja is a small stream running near river Perhonjoki draining its waters straight to the Baltic Sea north of the town of Kokkola. Korpilahdenoja is, however, considered part of the same Perhonjoki/Kälviänjoki river system, situated between river Perhonjoki and river Kälviänjoki. Korpilahdenoja is one of the largest lowland brooks in the

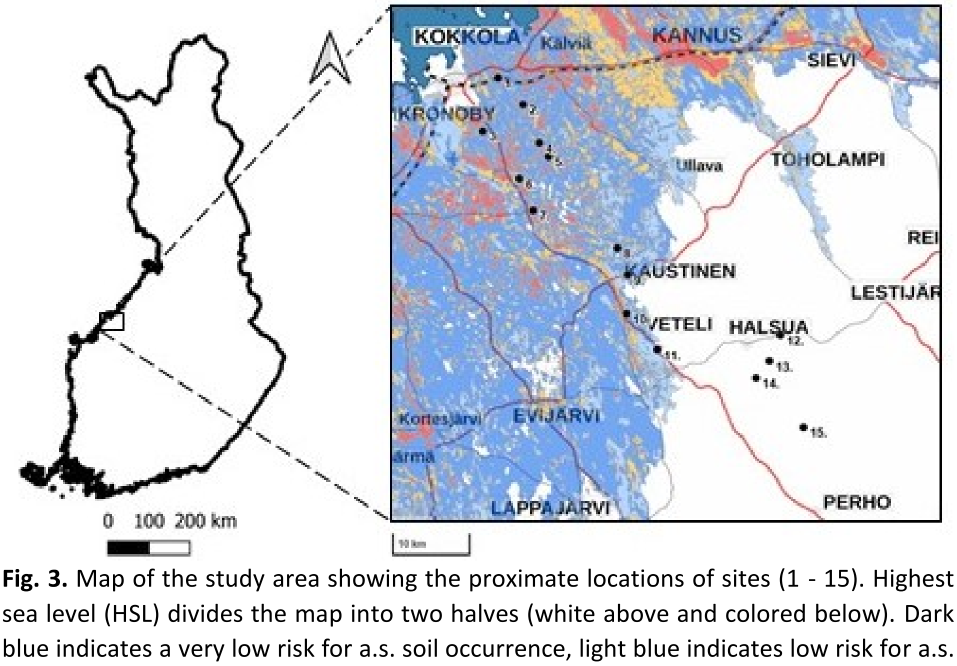

Fig. 3. Map of the study area showing the proximate locations of sites (1 - 15). Highest sea level (HSL) divides the map into two halves (white above and colored below). Dark blue indicates a very low risk for a.s. soil occurrence, light blue indicates low risk for a.s. soil occurrence, orange indicates risk for a.s. soil occurrence and red indicates high risk for a.s. soil occurrence. (NLS 2019)

area and that is why it was chosen for this study. All except one of the streams chosen are small (low order streams with catchment areas ≤ ~100 km^2). The exception is river Penninkijoki, which is located at the higher reaches of the river Perhonjoki catchment area. Penninkijoki was chosen for comparison because it runs high up in the system above the highest sea level in a fairly pristine environment. Higher reaches of river Penninkijoki is also quite small and the river size resembles some of the smaller streams studied in this thesis.

Table 1. Studied streams with *site number, site abbreviation, municipality, approximate altitude, situation towards HSL* and *approximate coordinates* (ETRS-TM35FIN). *Kainobäcken and Viitavesibäcken are part of the same stream but the name changes midstream from Kaino- to Viitavesibäcken. **Tastulanoja and Kalavedenoja are part of the same stream but the name changes to Kalavedenoja above lake Tastulanjärvi.

Site	Stream	Abbreviation	Municipality	Altitude (m)	Above or below (HSL)	Coordinates N	Coordinates E
1.	Korpilahdenoja	KO1	Kokkola	4	Below	7085247	317007
2.	Korpilahdenoja	KO2	Kokkola	13	Below	7080809	321225
3.	Åivobäcken	ÅI1	Kokkola	13	Below	7076301	314465
4.	Hömossadiket	HÖ1	Kokkola	23	Below	7074287	323990
5.	Hömossadiket	HÖ2	Kruunupyy	30	Below	7071985	325503
6.	Kainobäcken*	KAI1	Kruunupyy	25	Below	7068260	320575
7.	Viitavesibäcken*	VI1	Kruunupyy	40	Below	7062925	322959
8.	Tastulanoja**	TA1	Kaustinen	60	Below	7056559	337061
9.	Kalavedenoja**	KAL1	Kaustinen	78	Below	7052120	338809
10.	Saarivedenoja	SA1	Veteli	63	Below	7045589	338733
11.	Lammasoja	LA1	Veteli	88	Below	7039512	343774
12.	Kivioja	KI1	Halsua	128	Above	7041918	364455
13.	Kaihianoja	KA1	Halsua	130	Above	7040534	362703
14.	Puro	PU1	Halsua	123	Above	7034726	360404
15.	Penninkijoki	PE1	Halsua	166	Above	7026422	368406

Sites 1–11 were situated within the limits of HSL and sites 12–15 were situated above HSL. The sites ranged in altitude from < 5 m to approximately 165 m. The distance between the lowest site (site 1: Korpilahdenoja) and the highest site (site 15: Penninkijoki) was approximately 80 km. A straight cross-section of the topography in the study area shows a steady rise in altitude from coast (site 1) to inland areas (site 15; Fig. 4)

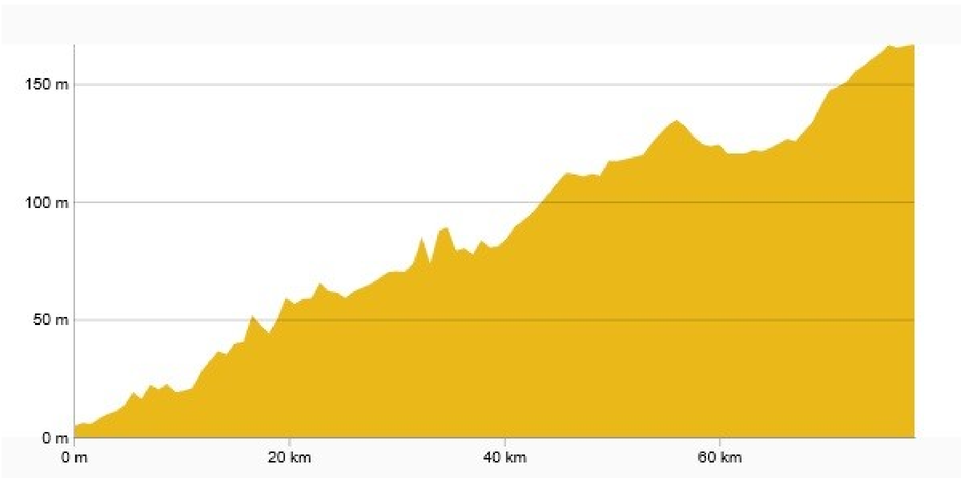

Fig. 4. A straight cross-section of the study area´s topography from site 1 (KO1) to site 15 (PE1). The altitude rises steadily from approximately 4 m at Korpilahdenoja to a little above 165 m at Penninkijoki. The HSL is situated between 40-50 km inland from site 1. (NLS 2019)

3.2 Water quality data

Water samples were collected on three occasions (twice in autumn 2018 and once in spring 2019) from all fifteen sites. pH-values and conductivity were measured from all sites at all occasions. Metals, sulfate and total organic carbon (TOC) were analyzed from all sites twice in autumn 2018. Metals were also analyzed from ten selected sites in spring 2019. pH values and conductivity were analyzed in laboratory with handheld pH/conductivity meter. Metals were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). Sulphur concentrations were analyzed at Åbo Akademi University Department of Organic Chemistry with ion chromatography (IC). TOC was analyzed using Shimadzu TOC-V CSN.

3.3 Invertebrate sampling

Invertebrate sampling was conducted between 25th and 27th September 2018 in all the fifteen sites with a kick-net sampler with a mesh size of 0.5 mm. Three parallel samples were collected from all sites. Normally, the number of parallel samples should be four, but the streams were small, and it was difficult to find four similar and intercomparable sites from all the study sites. For that reason, only three parallel samples were collected from all sites consisting of two samples from areas with small to medium-sized substrate and one from areas with larger substrate and boulders.

Samples were collected by kicking the substrate in front of the kick-net for thirty seconds and moving approximately 1 m upstream during that time. Collected material was sieved through a sieve with the mesh size of 0.5 mm and transferred to a sealed plastic container with the volume of 0.5 L. All larger branches and pebbles were thoroughly washed in the sieve before thrown away to ensure that as many invertebrates as possible where collected in the sample. The samples were then preserved in 70% ethanol for further analyses.

The species in the samples were analyzed, and individuals were counted under a microscope. Individuals were determined down to species level, if possible, using taxonomical literature. However, many of the species were difficult to determine with certainty, because of insufficient identification literature and time. Therefore, individuals are determined to family levels in this thesis. Individuals from the classes *Oligochaeta* and *Nematoda* were left out of the analyses. Individuals of the caddisfly *Hydopsychidae* were determined to species level using identification keys from Vuori (2002). Small and difficult-to-determine individuals were determined as juvenile *Hydropsyche* and were left out from further analyses.

3.4 Trichoptera *Hydropsychidae* analyses

Different caddisfly species from the family *Hydropsychidae* were given values according to their tolerance toward pollutants (Table 2). Values ranged from one (1) to four (4), where one was the value for tolerant species and four the value for sensitive species. Values for the species were taken from Vuori (2002).

Table 2. Different sensitivity values for different *Hydropsyche* species ranging from 1 to 4. *H. angustipennis* is the least sensitive species with the lowest value (*1*). *H. pellucidula* is more sensitive with a value of *2*. *H. saxonica* and *H. siltalai* are the most sensitive species and both are ordered sensitivity value *4*. Values collected from Vuori (2002).

Species	**H. angustipennis**	**H. pellucidula**	**H. Saxonica**	**H. siltalai**
Value (1-4)	1	2	4	4

A sensitivity value was given by calculating the mean value of the sum of all individuals and species for all the sites.

$$Sensitivity\ value = \frac{n(angustipennis * 1) + n(pellucidula * 2) + n(saxonica * 4) + n(siltalai * 4)}{n\ Hydropsychidae\ in\ sample}$$

Sites with mainly tolerant species were given a low value, whereas sites with mainly sensitive species had a higher value. Values from each site were used in further analyses to compare stream *Hydropsychidae* communities.

Gill abnormalities were studied from every *Hydropsychidae* individual under microscope (Fig. 5). All individuals were classified according to healthy (no gill abnormalities), slightly abnormal (at least one of the gills reduced or damage in gill tuffs) and abnormal (severe darkening/reduction of gills and gill tuffs). Only individuals with severe darkening were used in further analyses and calculations. The *Hydropsychidae* gill abnormality value was determined by calculating the percentage of individuals with severe abnormalities in the sample.

$$gill\ abnormality\ value = \frac{n\ severe\ abnormal}{n\ Hydropsychidae\ in\ sample} * 100$$

Fig. 5. The difference between *two Hydropsyche* larvae from Korpilahdenoja brook. The individual to the left show healthy and lush gill tuffs, whereas the individual to the right show signs of abnormalities with darkened and reduced gill tuffs. The individual to the left was categorized as "*healthy*", whereas the individual to the right was categorized as "*abnormal*".

3.5 Statistical analyses

Water quality and taxonomical data showed non-normal distribution with some outliers. The data was analyzed statistically using Spearman's rank correlation test, NMDS with Bray-Curtis dissimilarity test and Mann-Whitney U-test (Wilcoxon in R). Due to small sample size, only maximum and minimum concentrations were used in the analyses. The study sites were divided into two groups according to their maximum sulfate concentration. The sites grouped within "Low" sulfate group had maximum sulfate concentrations of approximately < 3.5 mg/l. The sites grouped within "High" sulfate group had all maximum sulfate concentrations > 10 mg/l. The median sulfate concentration in Finnish streams is 3.5 mg/l (Lahermo et al. 1995). That is why the maximum sulfate concentration of approximately 3.5 mg/l was chosen as a divider between these two groups.

The species composition was compared between high- and low sulfur concentration stream sites. The stress of NMDS models was checked and kept under 0.1. An ANOVA was used on the NMDS model variances to ensure that the model plots represented real data. The ANOVA of model variances compared with actual obtained data variances had a p-value > 0.05 indicating that model plots did not differ significantly from real data. The species composition was illustrated by conducting a Detrended Correspondence Analysis (DCA) over found species for each site. DCA is often used in ecology to illustrate patterns or differences between species composition at different sites or habitats. More information about the use of DCA is found in e.g. Parnell and Waldren (1996) and Garono et al. (1996). All the analyses except DCA were performed with R (version 3.6.1.).

4 Results

4.1 Water quality

Water quality in the study area remained decent at most of the sites through the entire study period. However, there was a small drop in pH values and a rise in metal concentrations and EC in spring 2019, especially at the lowland streams. Minimum pH values ranged from 4.1 to 6.5, maximum EC ranged from 31 to 328 µS/cm, maximum Al concentration ranged from 65 to 3617 µg/l, maximum TOC ranged from 11 to 42 mg/l and maximum sulfate concentration ranged from 0.72 to 23.32 mg/l in the studied streams. The lowest sulfate concentrations were measured at sites above HSL, whereas the highest sulfate concentrations occurred at sites close to the coast. The highest aluminum concentrations were also found at lowland streams below HSL. The lowest minimum pH values were measured at sites below HSL, but sites such as Viitavesibäcken, Kainobäcken and Tastulanoja had minimum pH values > 6.0, even though they are situated below HSL. Maximum sulfate concentration showed a clear divide between high altitude (above ~ 90 m) and low altitude (under ~ 90 m) streams and the sites are divided into low or high sulfate concentration according to their maximum sulfate concentration. TOC values did not show any pattern between altitude, and the concentration varied both below and above HSL (Table 3).

Table 3. The results from water quality analyses. Results are shown in maximum (SO4, EC, Al and TOC) and minimum (pH) values. Site grouping (high or low) according to sulfate concentration is also shown in the table.

Site	Stream	Abbreviation	Sulfate group	Min pH	SO4 mg/l	EC µS/cm	Al µg/l	TOC mg/l
1.	Korpilahdenoja	KO1	High	5.3	19.27	154	758	25
2.	Korpilahdenoja	KO2	High	5.2	10.17	124	758	23
3.	Åivobäcken	ÅI1	High	4.1	22.66	328	3617	42
4.	Hömossadiket	HÖ1	High	5.3	15.70	100	979	17
5.	Hömossadiket	HÖ2	High	5.2	15.25	120	1116	17
6.	Kainobäcken*	KAI1	High	6.1	23.32	144	447	21
7.	Viitavesibäcken*	VI1	High	6.3	15.17	120	236	21
8.	Tastulanoja**	TA1	High	6.0	19.18	105	426	20
9.	Kalavedenoja**	KAL1	Low	5.8	1.52	31	401	22
10.	Saarivedenoja	SA1	High	6.0	10.26	96	123	14
11.	Lammasoja	LA1	Low	5.6	3.24	43	189	27
12.	Kivioja	KI1	Low	6.0	0.75	50	116	30
13.	Kaihianoja	KA1	Low	5.8	3.68	37	139	25
14.	Puro	PU1	Low	6.5	2.02	46	65	11
15.	Penninkijoki	PE1	Low	6.1	0.72	31	89	17

Water quality analyses showed a strong correlation ($p < 0.001$, $r_S = -0.81$) between altitude and maximum sulfate concentration (Fig. 6) and with altitude and maximum aluminum concentration ($p < 0.001$, $r_S = -0.87$). Both sulfate- and aluminum concentrations dropped with increasing altitude. There was a significant correlation ($p < 0.001$, $r_S = -0.89$) with the maximum EC value increasing with decreasing altitude and a weak but significant correlation ($p = 0.024$, $r_S = 0.58$) between rising altitude and increasing minimum pH value. There was a strong correlation with maximum sulfate concentration and maximum aluminum concentration ($p = 0.001$, $r_S = 0.76$) and with maximum sulfate concentration and EC ($p < 0.001$, $r_S = 0.861$). Both maximum aluminum concentration and EC rise with a rising maximum sulfate concentration. However, there was no significant correlation ($p = 0.228$, $r_S = -0.33$) between maximum sulfate concentration and minimum pH value.

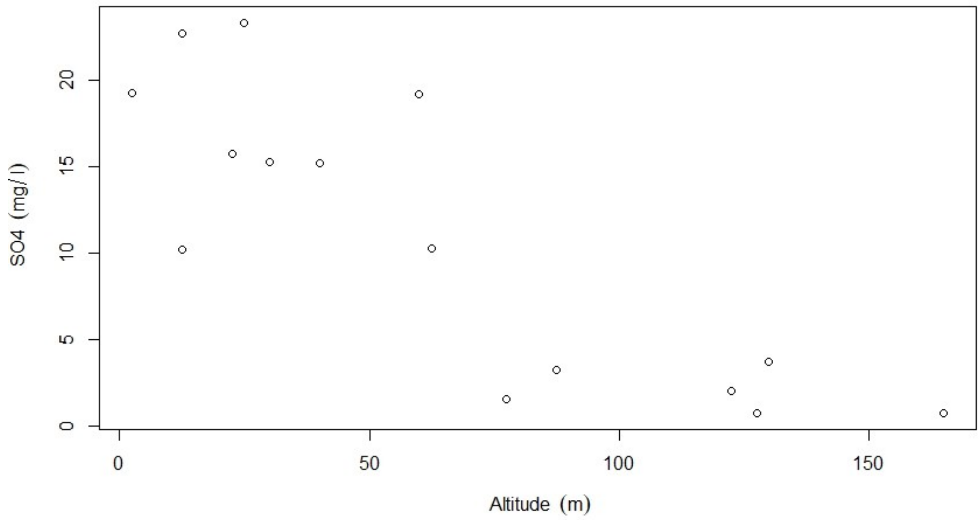

Fig. 6. The change in maximum sulfate concentration in mg/l (y-axis) with increasing altitude in meters (x-axis). The sulfate concentration decrease with increasing altitude ($p < 0.001$***, $r_S = -0.81$).

4.2 Species composition and *Hydropsyche* abnormalities

Stream riffles are inhabited with a varying range of different invertebrates. These invertebrates are divided into different classes of taxonomical levels ranging from species to families and from families to orders etc. Caddisflies e.g. could be ranked at different taxonomical levels from species to kingdom: species = *Hydropsyche siltalai*, genus = *Hydropsyche*, subfamily = *Hydropsychinae*, family = *Hydropsychidae*, superfamily = *Hydropsychoidea*, order = *Trichoptera*, class = *Insecta*, phylum = *Arthropoda* and finally kingdom = *Animalia*. This study shows species at species level (only from the genus *Hydropsyche*), family level and on order level.

Thirteen different taxonomical orders (Table 4) were obtained from the sites in this study (excluding individuals from the classes *Oligochaeta* and *Nematoda*). The counted individuals were from the orders *Arhynchobdellida*, *Coleoptera*, *Diptera*, *Ephemeroptera*, *Hemiptera*, *Hygrophila*, *Isopoda*, *Littorinimorpha*, *Megaloptera*, *Odonata*, *Plecoptera*, *Trichoptera* and *Veneroida*. The most diverse order was *Diptera* which included individuals from nine different families. These families were *Athericidae*, *Ceratopogonidae*, *Chaboridae*, *Chironomidae*, *Empididae*, *Pediciidae*, *Simuliidae*, *Tabanidaea* and *Tipulidae*. The order *Trichoptera* was almost as diverse as *Diptera* with individuals found from eight different families. These *Trichoptera* families included individuals from *Hydropsychidae*, *Hydroptilidae*, *Leptoceridae*, *Limnephilidae*, *Phryganeidae*, *Polycentropodidae*, *Psychomyiidae* and *Rhyacophilidae*. Other orders had individuals from only one or a few families.

Chironomidae (Diptera) was the most abundant family, with 5484 counted individuals. *Chironomidae* larvae dominated the species composition at six locations. These locations were Penninkijoki, Kivioja, Puro, Lammasoja, Tastulanoja and Åivobäcken. *Nemouridae* (Plecoptera) was the second most abundant family in this study with 4467 counted individuals. *Nemouridae* larvae dominated the species composition at seven locations. These locations were Kaihianoja, Korpilahdenoja site 1 and site 2, Viitavesibäcken, Hömossadiket site 1 and site 2 and Kainobäcken. *Polycentropodidae* (Trichoptera) larvae dominated the species composition at Kalavedenoja and *Simuliidae* (Diptera) were the most abundant species family in Saarivedenoja. Viitavesibäcken had the most counted individuals, whereas Åivobäcken had the fewest counted individuals in this study. *Hydropsychidae* larvae were found only at seven locations. These were Penninkijoki, Kalavedenoja, Korpilahdenoja site 1 and site 2, Tastulanoja, Viitavesibäcken and Kainobäcken.

NMDS (Nonmetric Multi-Dimensional Scaling) showed that there was a significant ($F = 2.904$, $Df = 14$, $p = 0.029$) difference in species composition on order level between streams with low sulfate concentration compared to streams with high sulfate concentration (Fig. 7a). Further analyses of EPT taxonomical groups showed that there was a significant ($F = 16.271$, $Df = 14$, $p = 0.004$) difference in Ephemeroptera species composition between high- and low maximum sulfate concentration streams (Fig. 7b). However, there was no significant difference in Trichoptera and Plecoptera species composition between the grouped streams. The results indicated that Ephemeroptera communities differed significantly within *Beatidae* ($Z = 78.947$, $W = 42$, $p = 0.010$) and *Lepthophlebiidae* ($Z = -78.947$, $W = 3$, $p = 0.010$) species composition (Fig. 8). The streams with high sulfate concentration had a significantly higher abundancy of *Beatidae* individuals compared to low sulfate streams, whereas low sulfate streams had more abundant *Lepthoplebiidae* populations.

The detrended correspondence analysis (DCA) show a distinctive grouping of sites corresponding with altitude (Fig. 9). The eigenvalue for AX1 was 0.414 and 0.120 for AX2 meaning that AX1 and AX2 combined explains more than 50 % of the variance between sites. The streams with high sulfate concentration tend to group to the left side of the graph, the streams just below and above 100 m altitude group in the middle, whereas Penninkijoki is situated far to the right. Taxa such as *Heptageniidae*, *Perlodidae*, *Leptophlebiidae* and *Hydroptilidae* are found far to the right in the graph, whereas *Nemouridae*, *Beatida* and *Simuliidae* are found at the opposite side. The result from DCA suggest that these taxa do not thrive in the same locations because of differences in environmental preferences.

Table 4. All the species counted at each site. The species are presented at order and family taxonomical levels. Individuals from the classes *Oligochaeta* and *Nematoda* are not counted in the table. Odanata are only assigned to order level. Species are shown to the left in the table, whereas sites are sorted by their approximate altitude from lowest to highest and named with their abbreviate name. See list of site abbreviations in Table 3.

Order	*Family*	*KO1*	*KO2*	*ÅI1*	*HÖ1*	*KAI1*	*HÖ2*	*VI1*	*TA1*	*SA1*	*KAL1*	*LA1*	*PU1*	*KI1*	*KA1*	*PE1*
Arhynchobdellida	*Erpobdellidae*								2		10					
Coleoptera	*Elmidae*		52		1	2		7	8		6		2	203	24	67
	Dysticidae		2	4	1						1	18	2	2		
	Haliplidae															5
Diptera	*Athericidae*								1							
	Ceratopogonidae		3		17	32	26	24	64	4	7	30	6	39	1	1
	Chaboridae			2												
	Chironomidae	214	694	32	125	321	118	455	780	220	220	606	497	373	94	735
	Empididae								6	4	2	1	3			
	Pediciidae	1	34	1	8	7	27	5	4	32	6	21	5	6	10	4
	Simuliidae	24	236		385	419	179	392	12	249	109	309	17	111	39	9
	Tabanidaea				3	4				1		2		14	4	9
	Tipulidae		6		1	1		1		2	1	1				3
Ephemeroptera	*Baetidae*	75	509	9	11	21	5	111	11	6	4	9				4
	Heptageniidae		7			1										29
	Leptophlebiidae	20	27			1		4	7		1	3		44	2	300
Hemiptera	*Corixidae*		1	10								1	1			
Hygrophila	*Planorbidae*			2	2										2	
Isopoda	*Asellidae*	89	160	16	2	76		35	8		1	61	40	20	7	6
Littorinimorpha	*Bithyniidae*									1						
Megaloptera	*Sialidae*	7			1	1		2								14
Odonata	*NA*		2	1		4		2	1	4		2		2	1	2
Plecoptera	*Leuctridae*	8	11		1	348		215	129	16	156			84		64
	Nemouridae	253	1016	25	859	488	428	763	73	57	96	283	13	14	46	53
	Perlodidae		3							1	9					78
Trichoptera	*Hydropsychidae*	23	114			69		1	4		62					18
	Hydroptilidae		2													318
	Leptoceridae										9					
	Limnephilidae	6	1	13	1	5		3	6	34	13	60	28	20	7	18
	Phryganeidae						1									
	Polycentropodidae	6	7		3	4	11	15	221	1	288	27	10	4	5	57
	Psychomyiidae					4		3	7		16		5	4		23
	Rhyacophilidae		14			16		50	7	1	10					10
Veneroida	*Sphaeriidae*	3	13						7			1				1
	Total	729	2914	115	1421	1824	795	2088	1358	633	1027	1435	629	940	242	1828

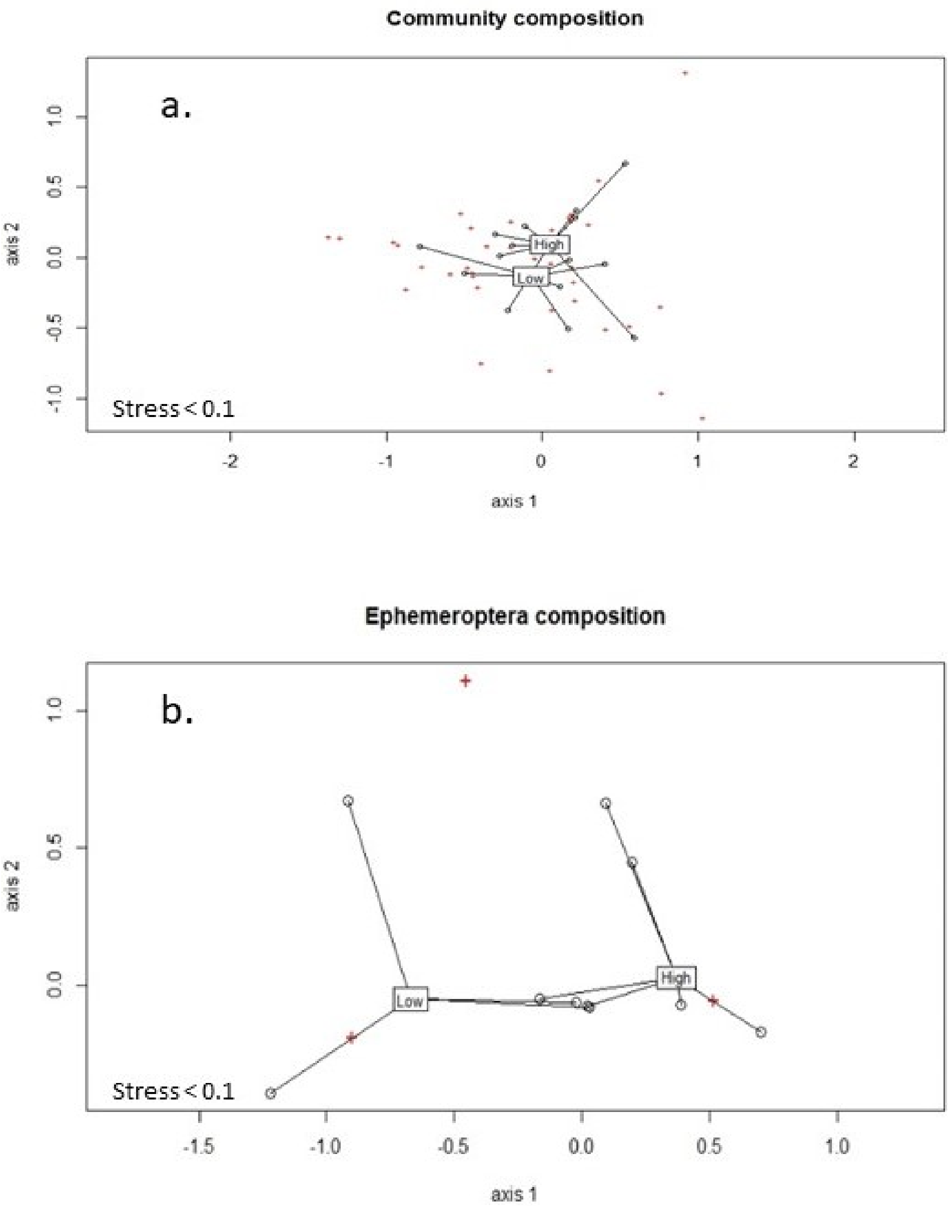

Fig. 7. Differences in species composition with NMDS Bray-Curtis dissimilarity at total community level (a.) and within Ephemeroptera taxonomical group (b.) between "*High*-sulfate" and "*Low*-sulfate" streams. There are some overlap at total community level, but the two groups differ from each other significantly ($p < 0.05$*). Difference between Ephemeroptera composition was significant ($p = 0.01$*) with less overlap.

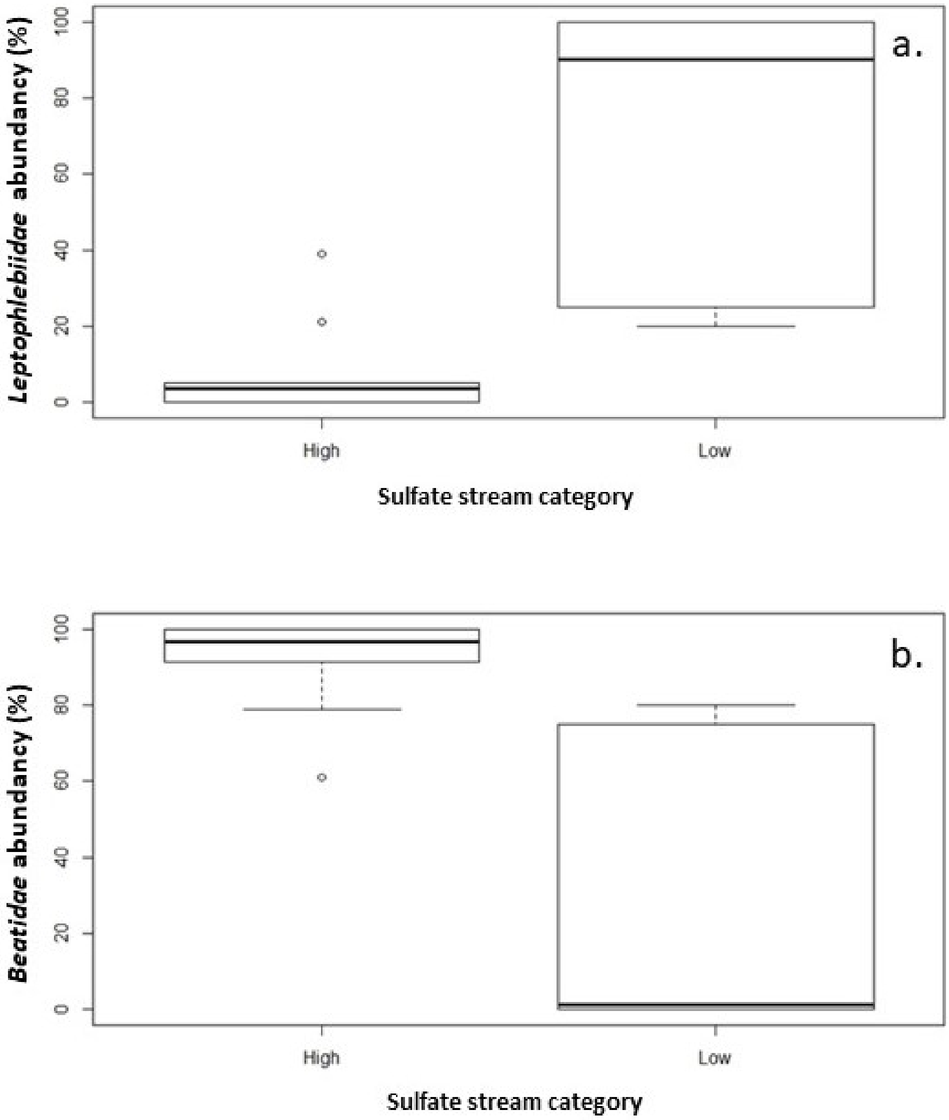

Fig. 8. Abundancies in percent (y-axis) *Leptophlebiidae* (a.) and *Beatidae* (b.) of total Ephemeropteran assemblages in "*High*-sulfate" and "*Low*-sulfate" streams (x-axis). High-sulfate streams have less variation in abundancies, whereas Low-sulfate streams have a larger within group variation. There is less than a minor overlap between these stream groups and the difference in both *Leptophelbiidae* ($p < 0,05^*$) and *Beatidae* ($p < 0,05^*$) abundancies are significant.

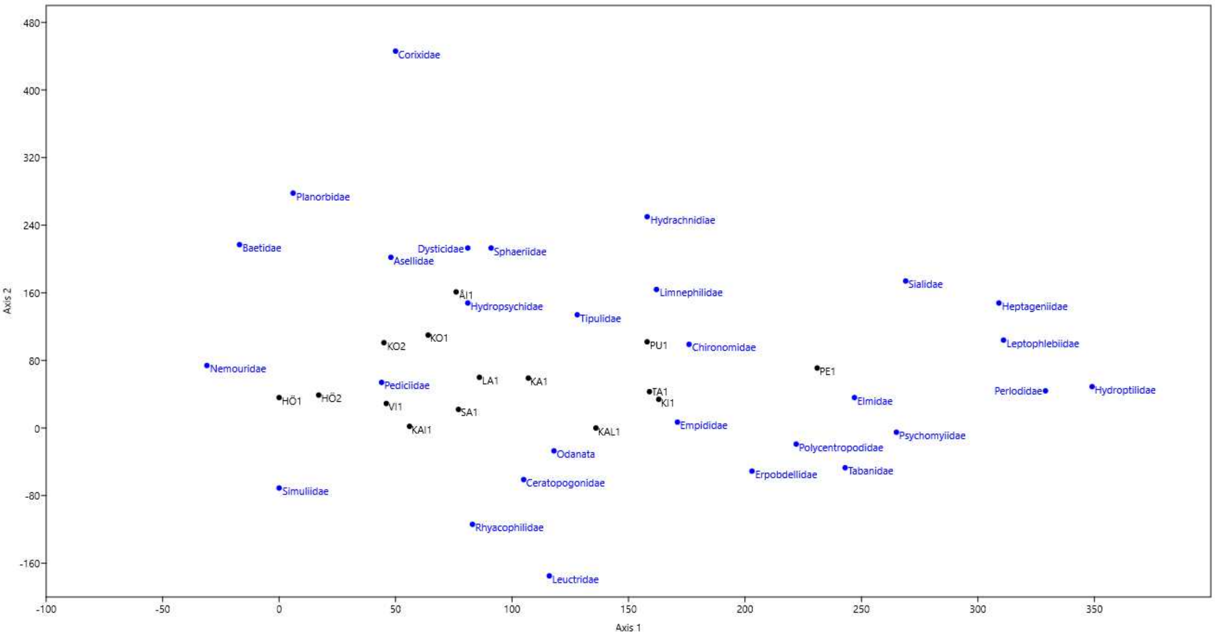

Fig. 9. Detrended correspondence analysis (DCA) of taxa and localities (sites). The eigenvalue for AX1 was 0.414 and 0.120 for AX2. DCA show three groupings; lowland (or "*High*-sulfate") mainly group to the left, highland (or "*Low*-sulfate") group to the middle and Penninkijoki as an own group on the right flank. Sites to the left group well with e.g. *Nemouridae*, *Simuliidae* and *Asellidae*, whereas Penninkijoki groups with e.g. *Hydroptilidae*, *Heptageniidae* and *Elmidae*.

Hydropsyche larvae were found only at seven locations with only one individual found at Viitavesibäcken brook. The *Hydropsyche* species consisted of *H. angustipennis*, *H. pellucidula*, *H.saxonica* and *H. siltalai*. Small and hard-to-identify young larvae were placed under juvenile *Hydropsyche* larvae (13 individuals in total). The most common species was *H. pellucidula* (128 observed individuals), which was found at all locations except Kalavedenoja. *Hydropsyche pellucidula* was the most common species of *Hydropsyche* at Korpilahdenoja (site 1 and site 2), Kainobäcken, Viitavesibäcken and Penninkijoki. *Hydropsyche siltalai* was observed at four locations and was the second most counted species with 75 individuals. Most of *H. siltalai* individuals were found at Kalavedenoja, where the species was the only *Hydropsyche* species identified. *Hydropsyche angustipennis* was found at all locations except Viitavesibäcken and Kalavedenoja. It was the third most counted *Hydropsyche* species with a total of 65 individuals. *Hydropsyche saxonica* was the scarcest *Hydropsyche* species with only 11 counted individuals found at Korpilahdenoja, Kainobäcken and Penninkijoki (Table 5).

The frequency of gill abnormalities varied between 0 and 13% of the counted individuals at different sites. The highest frequency of individuals with abnormal gills was found at Korpilahdenoja, whereas all the individuals at Viitavesibäcken, Tastulanoja and Penninkijoki were healthy. The difference in gill abnormality frequencies between sites with low sulfate concentration compared to sites with high sulfate concentration was non-significant. *Hydropsyche* species sensitivity values ranged between 1.5 and 4.0 within sites. The difference in sensitivity values was small and non-significant between sites. There was also no significant difference between *Hydropsyche* species composition at high- and low sulfate concentration streams.

Table 5. All the observed *Hydropsyche* species, counted juvenile individuals, sensitivity value (1 – 4) and frequency of gill abnormalities in individuals within sites.

Site	Stream	*H. angustipennis*	*H. pellucidula*	*H. saxonica*	*H. siltalai*	Juv. *Hydropsyche*	Tot include Juv.	Exclude Juv.	Sensitivity index	Abnormalities (%)
1.	Korpilahdenoja	5	16	0	0	2	23	21	1.8	13
2.	Korpilahdenoja	38	60	8	3	5	114	109	1.9	12
6.	Kainobäcken	18	35	2	10	4	69	65	2.1	1.4
7.	Viitavesibäcken	0	1	0	0	0	1	1	2.0	0.0
8.	Tastulanoja	2	2	0	0	0	4	4	1.5	0.0
9.	Kalavedenoja	0	0	0	60	2	62	60	4.0	1.6
15.	Penninkijoki	2	13	1	2	0	18	18	2.2	0.0

5. Discussion

Year 2018 was exceptionally dry in the area, which led to drought especially in the higher reaches of the river Perhonjoki catchment area. Spring 2018 (March, April and May) was especially dry with only an average of 27.2 mm precipitation per month in Halsua and 19.7 mm precipitation per month in Kaustinen. The average monthly precipitation in spring between the years 2010 and 2017 has been 56.7 mm for Halsua and 53.7 mm for Kaustinen. The same trend continued through the summer of 2018, with Halsua receiving approximately 10 mm less precipitation per month than normal. Precipitation for Kaustinen was lower than normal in the months of June and July, but the area was hit by heavy thunderstorms in August, raising the precipitation to above average for the summer months of 2018. Autumn (September, October and November) continued exceptionally dry in the region. The average precipitation in Halsua was 46.4 mm per month and only 39.1 mm per month in Kaustinen. These are much lower compared to the average autumn precipitation between the years 2010 and 2017. The average monthly autumn precipitation in Kaustinen has been 52.3 mm and 55.7 mm in Halsua (FMI 2019).

5.1 Water quality

The drought led to situations were only a low baseflow was noticeable, especially in streams around the Halsua area. This led to problems with water quality monitoring and one of the planned samplings for autumn 2018 was conducted in the following spring 2019. Problems with water quality in acid sulfate soil-affected streams are usually noticeable under high flows, when leached metals and sulfuric acid wash out to the streams (e.g. Toivonen et al. 2019). Acid sulfate soil-affected streams in Central-Western Finland usually show signs of severe chronical or seasonal acidity (pH often below 5.5), high EC, and high metal concentrations (e.g. Roos & Åström 2005; Åström & Björklund 1996; Åström 2001; Saarinen et al. 2010). The lack of high flow occasions during the monitoring period made identification of acid sulfate soil impact on streams more difficult. The water quality in acid sulfate soil-affected streams was unusually good with lower metal concentrations and higher pH values in autumn 2018 than expected. This was due to the lack of high runoff episodes that usually occur in spring and after autumn rainfall. However, there was a significant correlation with sulfate concentration, aluminum concentration and EC rising towards the coast. The EC value increased from ≤ 50 µS/cm at locations with altitude above 85 m to ≥ 100 µS/cm at locations below the altitude of 85 m. These EC values are low compared to e.g. Åström & Björklund (1996), who measured median EC values as high as 490 µS/cm (n = 130) under high autumn water flow in small streams draining the Munsala river basin. Even if the EC values are lower in this study, they do differ between high and low altitude streams, indicating a shift in acid sulfate soil occurrence at the altitude of approximately 85 m above sea level.

Sulfate is a highly soluble and conservative ion in water, which makes sulfate concentration a good indicator of acid sulfate soil-impact on stream water quality. Lahermo et al. (1995) monitored 1167 sampling sites and concluded that the median sulfate concentration in Finnish streams was 3.5 mg/l. The shift in sulfate concentration correlated well with the highest sea level of the Littorina Baltic Sea phase. All the locations with high sulfate concentration (above 3.5 mg/l) were situated below the highest shoreline, which indicates that sulfate has leached from acid sulfate soils, raising maximum sulfate concentration in coastal streams. This is especially apparent in streams that have a larger proportion of acid sulfate soils within their catchment area. Similar results have been obtained in earlier studies, where sulfate concentration rose with rising proportion of acid sulfate soils within stream catchment area (e.g. Beucher et al. 2015). Because of its persistent nature, sulfate concentration tends to increase also downstream within streams. This was noticeable in this study, where maximum sulfate concentration rose downstream within e.g. Kaino- and Viitavesibäcken stream.

Metals, such as aluminum, are highly enriched in streams affected by acid sulfate soils (e.g. Lahermo et al. 1995). A maximum aluminum concentration >1000 µg/l was only measured in Åivobäcken and Hömossadiket (site 2). High metal concentrations in these two small streams are well reported from earlier studies. Tolonen (2002) measured extremely high concentrations of aluminum in both Åivobäcken and Hömossadiket. Åivobäcken had an aluminum concentration of 12 300 µg/l (autumn 1997) and the aluminum concentration at Hömossadiket was 4 960 µg/l (autumn 1997). In this study, aluminum correlated significantly with the maximum sulfate concentration and with decreasing altitude. However, there were streams that had low concentration of aluminum, even if the sulfate concentration was high. The difference between Viitavesibäcken and Hömossadiket is a good example. The maximum aluminum concentration was 236 µg/l and maximum sulfate concentration was 15.17 mg/l at Viitavesibäcken. Hömossadiket (site 2) had a maximum aluminum concentration of 1116 µg/l and a maximum sulfate concentration of 15.25 mg/l. Even if the sulfate concentrations are almost identical, there is a significant difference in aluminum concentration between the two streams. The difference in aluminum concentration is due to differences in pH values. Viitavesibäcken had a minimum pH value of 6.3, whereas Hömossadiket (site 2) had a minimum pH value of 5.2. The difference in pH values between these two streams is something that have been noticed in previous studies, with pH values seldom dropping below 5.5 in Viitavesibäcken, whereas pH values drop below 5.0 every year at times with high water flow in Hömossadiket (Willner unpublished). Aluminum is known to be pH dependent and form soluble Al^{3+}-ions under acidic conditions. However, soluble aluminum tends to form insoluble hydroxysulfates, when mixed with water that has a higher pH value (e.g. Bigham & Nordstrom 2000). Acidic and metal-rich drainage water may form metal precipitates when mixing with more neutral and well-buffered water in Viitavesibäcken, keeping the metal concentration low compared to other streams in the area. This is something that needs more investigation in the future.

The minimum pH values ranged from 5.8 to 6.5 in low sulfate concentration streams, with average combined minimum pH being 6.0 (n = 6). The minimum pH values ranged from 4.1 to 6.3 in high sulfate concentration streams and the average combined minimum pH value for these streams was 5.5 (n = 9). Only Åivobäcken showed signs of severe acidity, with the pH value dropping to 4.1 in spring 2019. It is well reported that pH values usually drop in autumn after heavy rainfall in small streams heavily affected by acid sulfate soils (e.g. Åström & Björklund 1996). The small streams near the coast in this study are no exception, and as earlier mentioned, the pH value usually drops below 5.0 during high flows in Hömossadiket. Tolonen (2002) monitored e.g. Åivobäcken and Hömossadiket streams between the years 1997 and 2000. In his studies, pH values dropped as low as 4.1 in Hömossadiket (autumn 1997) and 3.9 in Åivobäcken (autumn 1997). This dramatic drop in pH values did not occur in autumn 2018, but did so in Åivobäcken in spring 2019.

The absence of high flow occasions during the monitoring period has surely affected the results. Water quality was decent in the studied streams compared to e.g. acid sulfate soil-affected streams within the Munsala river basin in Åström & Björklund (1996). However, there is a significant difference in maximum metal concentrations, minimum pH values, maximum sulfur concentrations and electric conductivity between lowland and highland streams. Even if the data set is small, and the pH values mostly stayed on decent levels, annual fluctuations must have a negative impact on invertebrates in the studied streams. This should be visible from the invertebrate data, which make invertebrates important bioindicators of acid sulfate soil-impact especially at times without high flows. Combining invertebrate data with water quality data should give a good picture of the most affected streams around the river Perhonjoki basin.

5.2 Effects on invertebrate communities

Small streams within the river Perhonjoki region are home to many invertebrate species. Heino et al. (2002) showed in their studies that streams within the river Perhonjoki ecoregion are mainly dominated by shredder- and filter-feeders (*Hydropsychidae* and blackflies). These are invertebrates that normally dominate small-sized (first to third order) streams in the area. A change in species composition could be the result of many factors that affect invertebrate communities. It is good to bear in mind that this survey covered a large area with different stream types with complex problems due to human activity in both low sulfate (non-affected) and high sulfate (acid sulfate soil-affected) streams. These problems are often caused by multi stressor effects (e.g. Sundermann et al. 2013; Tolkkinen 2015). These stressors are a combination of e.g. water quality, land use and habitat deterioration. Habitat deterioration is easily observable around lowland areas of the Perhonjoki river catchment area, where low reliefs have resulted in deeply dug channelized streams to enable cultivation of arable land. The upper reaches of the river system have been channelized and drained for e.g. timber floating and drainage of peatlands for forestry. Drainage and channelization of streams have made stream habitats more homogenous and the loss of vegetation cover has been huge. This is especially noticeable from moss cover in lowland streams. Sites that totally lacked moss cover was Åivobäcken, Hömossadiket (site 1 and 2), Korpilahdenoja (site 1 and 2) and Kainobäcken. All these sites were located below 40 m above sea level. Viitavesibäcken had sparse moss cover and all the rest had varying but significant cover of moss. Moss is an important factor shaping benthic communities. Koljonen et al. (2012) reported that leaf retention was lowest in channelized artificial streams without moss cover. The addition of moss significantly enhanced leaf retentiveness. Muotka and Syrjänen (2007) had similar results in their study. Their results indicated that moss cover was an important factor influencing leaf retention in boreal streams. Their results also indicated that channelized streams (or streams without moss cover e.g. after restoration) have a higher abundancy of scraper- and filter-feeders compared to shredder-feeding invertebrates, that are more abundant in more pristine streams. They implied that this difference could be because stones without moss are readily colonized by algae, which are used as food source for scraper- and filter-feeders. However, a low leaf retention results in scarce food resources for shredder-feeders, tilting the abundancy in the favor of scraper- and filter-feeders. That is why the absence of especially filter-feeders such as *Hydropsychidae* or *Simuliidae* in studied streams could indicate a change in normal species composition due to poor water quality.

There was a significant difference in species composition comparison between high- and low sulfate streams. A closer examination of the EPT-species showed that there was a significant difference in Ephmereoptera species between these two groups. The lowland streams with higher sulfate- and metal concentration were dominated by *Baetidae* species while *Leptophlebiidae* dominated in the streams with low sulfate concentration. This result was surprising, because e.g. *Baetis rhodani* is well known for drastically increasing mortality and stress at low (< 6.0) or very low (< 5.0) pH values (e.g. Andrén & Eriksson Wiklund 2013; Gerhardt 1990; Willoughby 1988; Ormerod et al. 1987). Willoughby (1988) suggested that *B. rhodani* might tolerate acidic circumstances if the water had a high ionic load, but *B. rhodani* was usually absent in streams with a pH value below 5.2. Results from Gerhardt (1990) indicate that *Lepthoplebiidae* would not be as sensitive toward a change in pH value, with *Leptophlebia marginata* showing no significant signs of changes in larvae survival with changing pH values and Cd stress. Gerhardt (1992a) showed that *Leptophlebia marginata* endured extremely acid (pH 4.5) conditions, but a combination of high iron concentration and low pH raised mortality rates at the end of a 30-day experiment. *Leptophlebia marginata* has proven to be more tolerant to Cd stress compared to *Beatis rhodani* in a laboratory experiment using both static- and flow-through systems (Gerhardt 1992b). Gerhardt (1995) reported that *L. marginata* individuals suffered from a change in ion balance (loss of Cl^- and K^+) when exposed to Cd. This led to inactivity and eventually death. Gerhardt also showed that Fe crusted on the larvae gill surface and in the gut membrane, leading to decreased food consumption and to starvation. Interestingly a high Fe concentration showed to counteract with Cd lowering the uptake of Cd in larvae. Even if *Leptophlebiidae* species are more tolerant to acidity, they do however show symptoms at low pH values. Gerhardt et al. (2005) reported that *Choroterpes picteti* (*Leptoblebiidae*) showed apparent symptoms at pH ≤ 5.0. Their results indicated that plain acid was worse than a combination of acid and acid mine drainage (AMD). From earlier studies, one could expect that *Beatidae* species would be absent or less abundant in acidic streams. It is good to remember that *Beatidae* is a large family consisting of both sensitive and tolerant species. All the *Beatidae* species found at Åivobäcken brook was of the species *Cloeon dipterum*, which is known to be one of the most tolerant *Beatidae* species (e.g. Lock & Goethals 2011). However, *Beatidae* species did thrive in lowland streams such as Korpilahdenoja (site 2) and Viitavesibäcken. These sites have proven to be less polluted by acid sulfate soils compared to other sites in the area (e.g. Hömossadiket). The nutrient-rich water, open farmland areas and low moss cover make these sites optimal for algae growth. This could be the reason to why *Beatidae* inhabited these sites in such large numbers.

The Ephemeropterans *Heptageniidae* were only found at three locations. These findings consisted of 29 individuals at Penninkijoki (1.6% of the total abundancy), seven individuals (< 0.5% of the total abundancy) at Korpilahdenoja (site 2) and only one individual (< 0.1% of total abundancy) at Kainobäcken. It is difficult to draw any conclusions regarding *Heptageniidae* abundancy because of the small data set. The *Heptageniidae* individuals were found at locations with heterogenous bottom substrate size (mixture of small pebbles and larger rocks/boulders) and higher than average water velocity in the study area. *Heptegeniidae* larvae usually live at riffles, where they scrape algae fixed on rocks or organic surfaces (e.g. Merritt et al. 2017). The DCA showed that *Heptageniidae* species grouped to sites with higher pH value, lower sulfate concentration and low aluminum concentration. Rainbow et al. (2012) reported that *Heptageniidae* species were absent or scarce where a Cu concentration in *H. siltalai* exceeded 170 µg Cu per g larval weight. This threshold concentration is much lower compared to a similar threshold concentration for *Beatidae* species (≥ 1000 µg Cu per g *H. siltalai* larval tissue in e.g. Awrahman et al. (2016)). Comparing results from this study with their results would imply that *Heptageniidae* individuals were absent or scarce in many lowland streams because of high metal bioavailability. More research is needed to rule out other factors, such as poor habitat as the main constraining factor shaping *Heptageniidae* communities at lowland streams within the river Perhonjoki catchment area.

There was no significant difference in Plecoptera species composition between the high- and low sulfate streams in this study. Rosemond et al. (1992) studied acidic streams in the United States and showed that there was no significant correlation between Plecoptera communities and pH nor aluminum. Findings from this study strengthen the idea that Plecoptera species are more tolerant to low pH than many other taxa. However, their studies did show a negative correlation with decreasing pH and a rising aluminum concentration on Trichoptera species. Results from this study did not show any significant difference between Trichoptera species in low- and high sulfate streams. This result was unexpected and contradict with earlier research. However, there was a noticeable difference in *Hydroptilidae* species abundancy between sites. *Hydroptilidae* individuals were found only at two locations (Penninkijoki and Korpilahdenoja site 2). The abundance of *Hydroptilidae* was as high as 17.4% at Penninkijoki, whereas only two individuals could be found at Korpilahdenoja (site 2). There was also a slightly noticeable pattern within *Psychomyiidae* abundancies. *Psychomyiidae* larvae were found in both high- and low sulfate streams. Not a single *Psychomyiidae* larva was found at Hömossadiket nor Åivobäcken streams. Trichoptera abundancies were low at these two streams compared with other streams. Even though the abundancies of Trichoptera species was low at these two sites, other streams with high sulfate concentration had many Trichopteran individuals. This difference within group made comparison of Trichopteran assemblages difficult between high- and low sulfate streams.

Species from the Trichopteran *Hydropsychidae* were found at Penninkijoki, Kalavedenoja, Tastulanoja, Viitavesibäcken, Kainobäcken and both sites at Korpilahdenoja. Four different *Hydropsyche* species were found in this study. The species *H. angustipennis*, *H. pellucidula*, *H. siltalai* and *H. saxonica*. *H. angustipennis* were found at all locations except Viitavesibäcken and Kalavedenoja streams. *Hydropsyche pellucidula* was found at all above-mentioned streams except Kalavedenoja. *Hydropsyche siltalai* was found at four locations and it was the only *Hydropsychidae* species found at Kalavedenoja. *Hydropsyche siltalai* was also found in Penninkijoki, where its abundancy was approximately 11% of the entire *Hydropsychidae* population. *Hydropsyche siltalai* abundancy of *Hydropsyche* populations were approximately 15% at Kainobäcken and only under 3% at Korpilahdenoja (site 2). *Hydropsyche saxonica* was found at Korpilahdenoja (site 2), Kainobäcken and Penninkijoki. *Hydropsyche saxonica* has been found at Tastulanoja in earlier studies of the stream (HERTTA 2020). However, no *H. saxonica* could be found during this study at Tastulanoja brook. Distribution of *Hydroppsyche* species followed predicted patterns. Most of the lowland *Hydropsychidae* species were *H. angustipennis*, which has been reported to be decently tolerant towards acidity and aluminum (e.g. Vuori 1996). *Hydropsyche siltalai* was mainly found higher up in the catchment area, which is in line with earlier research, where *H. siltalai* has been shown to decrease in lowland streams because of e.g. higher temperature, higher nutrient load and slower current (e.g. Basaguren & Orive 1990; Tolkamp & Higler 1983). Hildrew (1977) reported that *H. siltalai* thrive on stones in moss cover and that there was a significant positive correlation with *H. siltalai* abundancy with moss cover. However, the same correlation could not be found with *H. pellucidula* in his study. Difference in *H. siltalai* distribution could be a result of differences in acidity and aluminum concentration. Vuori (1996) showed that *H. siltalai* larvae were less tolerant to a heightened aluminum concentration and low pH values compared *to H. angustipennis*. This difference in sensitivity could well be the reason why *H. siltalai* is less abundant at lowland acid sulfate soil-affected streams.

Hydropsyche larvae were unfortunately absent at many sites above the highest sea level. This made advanced statistical analyses difficult between streams. It is difficult to pinpoint an exact reason to why *Hydropsychidae* species were absent in these streams. However, it is notable that the Halsua area was strongly affected by drought in the same year. This could be the reason for the disappearance of these species from the area. Their absence could also be due to errors in sampling and/or coincidence. It is also possible that the larvae were missed when sorting the material, as the small instar larvae are difficult to notice. This idea is supported by other studies, such as Sundermann et al. (2008), who reported that there were differences between two sites within the same stream, even though the sites were close to each other and almost identical in water quality and structure. However, their studies showed that differences within a stream were subtler than differences between two random streams. This subtle but significant difference led to a different species list and different species abundancies within stream sites. They argued that the difference between site 1 and site 2 could be the result of errors in sampling and spatial variability within a stream reach. Similar differences in taxa have been showed to occur due to human errors in sorting and identification (Haase et al. 2006). Mykrä et al. (2006) showed that there was a significant difference between the number of taxa caught with kick-net sampling and the time used for sampling. Individuals from rare taxa were often absent when sampling was conducted for a total time of 1 min. However, a prolonged sampling helped in collecting rare taxa, but the prolonged sampling was very time and labor consuming. The small streams in this study made it impossible to take more than three parallel samples with a total time of 1.5 min. However, this could explain why some taxa were absent in unexpected places.

Another factor that needs to be considered is the fact that problems with serious acidity usually occur at times with high flow (e.g. Toivonen et al. 2019). There were no such occasions during the study period because of the drought. This could have led to a situation where the water quality was better than normal in many of the lowland streams and only chronically acidic streams showed symptoms of acid sulfate soils. This could have been especially apparent at sites which suffer only mildly from acid sulfate soils and show symptoms only under severe acidic periods. Chronically acidic streams, such as Hömossadiket and Åivobäcken, have lethal conditions for sensitive species almost every year. There have been no severe acidic impulses in the area since 2006. This could have led to the fact that streams that might suffer from acidity only after a heavy drought period (Korpilahdenoja, Kainobäcken, Viitavesibäcken and Tastulanoja) have managed to sustain diverse invertebrate populations. These streams might have many years with good enough water quality for in-stream biota, but extreme conditions might induce an acidic impulse that destroys invertebrate communities. However, years with better water quality make it possible for species to recolonize the streams. Sundermann et al. (2011) showed that species recolonize restored stream reaches if there is a suitable population for recolonization within 0–5 km upstream. The same recolonization of an empty reach could be achieved at streams recovering from an acidic impulse. Hömossadiket and Åivobäcken are heavily influenced from acid sulfate soils throughout the stream, but Kainobäcken, Viitavesibäcken and especially Tastulanoja headwaters are of better quality compared to their lower reaches. These streams could have sanctuaries for species under acidic conditions and from where species could again recolonize the lower reaches when the water quality improves. MacNeale et al. (2005) reported that adult stoneflies could fly even as far as 600 m through forest from their site of emergence or even 800 m upstream following the stream channel. This fact makes e.g. Kainobäcken and Korpilahdenoja sites even in-reach for invertebrate colonization from the Perhonjoki river. These factors could possibly have influenced invertebrate communities, narrowing the difference in species composition between high- and low sulfate-streams.

5.3 *Hydropsyche* gill abnormalities

Hydropsychidae larvae have been used in many experiments to identify effects of metals on stream invertebrates. Vuori (1996) reported that a low pH value (5.0) and elevated aluminum concentrations lead to darkening of individual analpapillae and problems with the individuals' ormoregulatory processes. Ruuth (2017) showed that elevated cadmium concentrations also induced gill abnormalities in *H. pellucidula* larvae. Vuori and Parkko (1996) reported that *H. pellucidula* showed gill abnormalities (darkening of gill tuffs) subjected to organic pollution at river Kymijoki.

The frequency of gill abnormalities varied between 0 and 13% in this study. Highest frequencies of gill abnormalities were found at Korpilahdenoja and the lowest (0%) were found at Viitavesibäcken, Tastulanoja and Penninkijoki. Two percent of the individuals at Kalavedenoja and 1% of the individuals at Kainobäcken showed signs of darkened gill tuffs. The differences in *Hydropsyche* species abundancies within sites make it difficult to draw decisive conclusions about differences in gill abnormality frequencies. For example, the only individual that was found at Viitavesibäcken and all the four individuals at Tastulanoja were healthy and showed no signs of darkened gill tuffs. This small data set for both streams makes statistical analyses of gill abnormalities difficult and unreliable. Classification of abnormalities is personal, and the classification can vary greatly between persons, which makes it difficult to even compare results from different research. Salmelin et al. (2015) studied the variation in classification of abnormalities of the Dipteran *Chironomidae*. Their results showed that classification as “normal” or “abnormal” differed between experts and it was difficult to find a common agreement in what classified as abnormal.

6 Conclusion

Results from this study confirmed the hypothesis to be correct for acid sulfate soil impact on water quality. Sulfate concentrations, aluminum concentrations and EC values rise towards the coast and the lowest pH values where measured in lowland streams. Results indicated that acidity below HSL are due to drainage of acid sulfate soils. Acidity above HSL was due to humic acids from peatlands. This was noticeable from much lower sulfate concentrations above approximately 85 m above sea level.

Results from this study confirmed the hypothesis that pollution from acid sulfate soils alters invertebrate species composition. Acid sulfate soil-affected streams differed from non-affected streams in their species composition, even if the lack of high flow periods narrowed the differences between streams. Effects of acid sulfate soil pollution were especially apparent at Åivobäcken and Hömossadiket brooks. Acidity and metals dissolved from acid sulfate soils are factors that constrain invertebrate species richness the most in these two brooks. This was seen from low pH-values, high aluminum concentrations, absence of *Hydropsychidae*, or blackflies larvae, and the fact that the total individual count was lowest from all the places at Åivobäcken, even though these two brooks are also heavily channelized and lack moss cover. Results from e.g. Muotka and Syrjänen (2007) suggest that these two taxa should thrive in these streams because of suitable habitats and large surfaces for algae growth.

The difference in EPT-species composition between acid sulfate soil-affected and non-affected streams, was apparent within Ephemeroptera species composition. However, results did not indicate any significant difference in Trichoptera or Plecoptera species composition. Results from this study indicated that there were no significant differences in *Hydropsyche* species composition nor in the frequency of gill abnormalities between acid sulfate soil-impacted and non-impacted streams. However, even if there were no statistically significant differences in gill abnormalities, there was a noticeable rise in gill abnormality frequencies towards the coast. This is something that needs more research in the future.

Hydropsyche larvae could be considered as good bioindicators in small streams around the Perhonjoki catchment area. As Heino et al. (2002) suggested, these are usually found in Perhonjoki ecoregion. However, as this study showed, it is difficult to draw conclusions out from kick-net sampling data alone. The risk of human error in sampling etc. is too big and there is always a risk of not catching any of the target specimens in sampling. Therefore, it could be better to also conduct *in situ* toxicological tests with target species to minimize the risk of errors.

Both the years 2018 and 2019 were extremely dry in the region. Reports from the area in autumn 2019 show that acidic impulse from acid sulfate soils is more than likely to occur following these dry years. It is, therefore, important to conduct new studies from the region after the next acidic episode. Results from this study serve as a good reference and the possible absence of species at next sampling could indicate mortality due to chock from acidity and metals. It would be important to conclude *in situ* experiments with *Hydropsychidae*, *Heptageniidae* and *Leptophlebiidae* larvae in streams where these taxa were absent. Results from *in situ* toxicity tests would give more knowledge about the impact of bad water quality on these species in heavily polluted streams.

Acknowledgements

I want to thank the Drainage Foundation sr. and Perhonjokirahasto for funding this thesis. This study was made possible due to your support. I also want to thank my family who have supported and encouraged me through the entire project. I also want to thank all the personnel at Åbo Akademi University, who have helped me with this project.

References

Airiola S., Koivisto A-M., Mäenpää E., Mäensivu M., Pakkala J., Teppo A. & Westberg V. (2016), Raportteja **47:2016**, Perhonjoen ja Kälviänjoen vesienhoidon toimenpideohjelma 2016-2021, Etelä-Pohjanmaan Elinkeino-, liikenne- ja ympäristökeskus (in Finnish)

Allan J. D. & Castillo M. M. (2007), *Stream Ecology: Structure and Function of Running Waters*, second edition, Springer

Andersen T. & Klubnes R. (1983), The Life Histories of *Hydropsyche siltalai* Döhler, 1963 and *H. pellucidula* (Curtis 1834) (Trichoptera, Hydropsychidae) in a West Norwegian River, *Aquatic Insects*, **5**, Issue 1, pages 51–62

Andrén C.M. & Eriksson Wiklund A-K. (2013), Response of Gammarus pulex and Baetis rhodani to springtime acid episodes in humic brooks, *Science of The Total Environment*, Volumes **463–464**, pages 690–699

Awrahman Z. A., Rainbow P. S., Smith B. D., Khan F. R. & Fialkowski W. (2016), Caddisflies Hydropsyche spp. as biomonitors of trace metal bioavalability thresholds causing disturbance in freshwater stream benthic communities, *Environmental Pollution*, **216**, pages 739–805

Awrahman Z. A., Rainbow P. S., Smith B. D., Khan F. R., Bury N. R. & Fialkowski W. (2015), Bioaccumulation of arsenic and silver by the caddisfly larvae Hydropsyche siltalai and H. pellucidula: A biodynamic modeling approach, *Aquatic Toxicology*, **161**, pages 196–207

Badcock R. M. (1976), The distribution of the Hydropsychidae in Great Britain, Proc. of the first Int. Symp. on Trichoptera, 1974, Junk. The Hague, pages 49–58

Basaguren A. & Orive E. (1990), The relationship between water quality and caddisfly assemblage structure om fast-running rivers. The river Cadagua basin, *Environmental Monitoring and Assessment*, **15**, Issue 1, pages 35–48

Beucher A., Fröjdö S., Österholm P., Auri J., Martinkauppi A. & Edén P. (2015), Assessment of acid sulfate soil mapping utilizing chemical indicators in recipient waters, Bulletin of the Geological Society of Finland, **87**, pages 5–23

Bigham J. M. & Nordstrom D. K. (2000), Iron and Aluminum Hydroxysulfates from Acid Sulfate Waters, *Reviews in Mineralogy and Geochemistry*, **40**, Issue 1, pages 351–403.

van Breemen N. (1973), Soil forming processes in acid sulphate soils. In: Dost, H. (ed.), Acid Sulphate Soils, Proc. Int. Symp. on Acid Sulphate Soils. IILRI, Wageningen, **18**, pages 66–130

Bärlund I., Tattari S., Yli-Halla M. & Åström M. (2005), Measured and simulated effects of sophisticated drainage techniques on groundwater level and runoff hydrochemistry in areas of boreal acid sulfate soils, *Agricultural and food science*, **14**, Issue 1, pages 98–111

Covich A. P., Palmer M. A. & Crowl T. A. (1999), The Role of Benthic Invertebrate Species in Freshwater Ecosystems Zoobenthic species influence energy flows and nutrient cycling, *BioScience*, **49**, Issue 2, pages 119–127

Deng H., Åström M. & Björklund A. (1998), Geochemical and mineralogical properties of sulfide-bearing fine-grained sediments in Finland., *Environmental Geology* **36**, Issues (1-2), pages 37–42

dos Reis Oliveira P. C., Kraak M. H. S., Pena-Ortiz M., van der Geest H. G., & Verdonschot P. F. M. (2019 article in press), Responses of macroinvertebrate communities to land use specific sediment food and habitat characteristics in lowland streams, *Science of The Total Environment*, **703**, 10 February 2020

FMI (2019), Finnish Meteorological Institute, Open information, https://en.ilmatieteenlaitos.fi/ (viewed 17.12.2019)

Garono R. J., Heath R. T. & Hwang S-J., (1996), Detrended Correspondence Analysis of Phytoplankton Abundance and Distribution in Sandusky Bay and Lake Erie, *Journal of Great Lakes Research*, **22**, Issue 4, pages 818–829

Gerhardt A. (1990), Effects of subacute doses of cadmium on pH-stressed Leptophlebia marginata (L.) and Baetis rhodani pictet (insecta: Ephemeroptera), *Environmental Pollution*, **67**, Issue 1, pages 29–42

Gerhardt A. (1992a), Effects of subacute doses of iron (Fe) on *Leptophlebia marginata* (Insecta: Ephemeroptera), *Freshwater biology*, **27**, pages 79–84

Gerhardt A. (1992b), Acute toxicity of Cd in stream invertebrates in relation to pH and test design, *Hydrobiologia*, **239**, Issue 2, pages 93–100

Gerhardt A. (1995), Joint and single toxicity of Cd and Fe related to metal uptake in the mayfly Leptophlebia marginata (L.)(Insecta), *Hydrobiologia*, **306**, pages 229–240

Gerhardt A., Janssens de Bisthoven L. & Soares A. M. V. M. (2005), Effects of Acid Mine Drainage and Acidity on the Activity of Choroterpes picteti (Ephemeroptera: Leptophlebiidae), *Arch Environ Contam Toxicol*, **48**, Issue 4, pages 450–458

Haase P., Murray-Bligh J., Lohse S., Pauls S., Sundermann A., Gunn R. & Clarke R. (2006), Assessing the impact of errors in sorting and identifying macroinvertebrate samples, *Hydrobiologia*, **566**, Issue 1, pages 505–521

Heino J., Muotka T., Paavola R., Hämäläinen H. & Koskiniemi E. (2002), Correspondence between regional delineations and spatial patterns in macroinvertebrate assemblages of boreal headwater streams, *J. N. Am. Benthol. Soc.*, **21**, pages 397–413

HERTTA (2020), SYKE, Finnish Environment Institute, Open information, https://www.syke.fi/en-US/Open_information (viewed 13.1.2020)

Hildrew A. G. & Edington J. M. (1979), Factors facilitating the coexistence of Hydropsychid caddis larvae (Trichoptera) in the same river system, *Journal of Animal Ecology*, **48**, Issue 2, pages 557–576

Hildrew A. G. (1977), Ecological aspects of life history in some net-spinning Trichoptera, Proc. of 2nd Int. Symp. on Trichoptera, 1977, Junk, The Hague

Hutchens Jr. J. J., Wallace B. & Grubaugh J. W. (2017), Transport and Storage of Fine Particulate Organic Matter, *Methods in stream ecology*, **2**, chapter 25, pages 37–53

Joukainen S. & Yli-Halla M. (2003), Environmental impacts and acid loads from deep sulfidic layers of two well-drained acid sulfate soils in western Finland, *Agriculture, Ecosystems And Environment*, **95**, Issue 1, pages 297–309

Koljonen S., Louhi P., Mäki-Petäys A., Huusko A. & Muotka T. (2012), Quantifying the effects of in-stream habitat structure and discharge on leaf retention: implications for stream restoration, *Freshwater Science*, **31**, Issue 4, pages 1121–1130

Kowalik R. A. & Ormerod S. J. (2006), Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates, *Freshwater Biology*, **51**, pages 180–191

Lahermo P., Salminen R., Tarvainen T. & Väänänen P. (1995), Geochemical mapping of stream waters and sediments in Finland: Selected results, Geological Survey of Finland, Special Paper **20**, pages 155–166

Lenat D. R. (1988), Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates*, J. N. Am. Benthol. Soc*., **7**, Issue 3, pages 222–233

Lepori F. & Ormerod S. J. (2005), Effects of spring acid episodes on macroinvertebrates revealed by population data and in situ toxicity tests, *Freshwater Biology*, **50**, pages 1568–1577

Lock K. & Goethals P. L. M. (2011), Distribution and ecology of the mayflies (Ephemeroptera) of Flanders (Belgium*), Ann. Limnol. - Int. J. Lim*., **47**, Issue 2, pages 159–165

MacNeale K. H., Peckarsky B. L. & Likens G. E. (2005), Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors, *Freshwater Biology*, **50**, Issue 7, pages 1117–1130

Malas D. & Wallace B. J. (1977), Strategies for coexistence in three species of net-spinning caddisflies (Trichoptera) in second-order southern Appalachian streams, *CAN. J. ZOOL.*, **55**, pages 1829–1840

Malte H. & Weber R. E (1988), Respiratory stress in rainbow trout dying from aluminium exposure in soft, acid water, with or without added sodium chloride., *Fish Physiology and Biochemistry*, **5**, Issue 4, pages 249–256

Mattbäck S., Boman A. & Österholm P. (2017), Hydrogeochemical impact of coarse-grained post-glacial acid sulfate soil materials., *Geoderma*, **308**, pages 291–301

McLay C. L. (1968), A study of drift in the Kakanui river, New Zealand, *Aust. J. mar. Freshwat*. *Res*., **16**, pages 139–149

Merritt R. W., Cummins K. W. & Berg M. B. (2017), Trophic Relationships of Macroinvertebrates, *Methods in stream ecology*, **2**, chapter 20, pages 413–433

Muotka T. & Syrjänen J. (2007), Changes in habitat structure, benthic invertebrate diversity, trout populations and ecosystem processes in restored forest streams: a boreal perspective, *Freshwater Biology*, **52**, Issue 4, pages 724–737

Mykrä H., Ruokonen T. and Muotka T. (2006), The effect of sample duration on the efficiency of kick-sampling in two streams with contrasting substratum heterogeneity, *Verh. Internat. Verein. Limnol*., **29**, Issue 3, pages 1351–1355

NLS (2019), National Land Survey of Finland, open data, Paikkatietoikkuna, https://kartta.paikkatietoikkuna.fi/?lang=en (viewed 17.12.2019)

Nystrand M., Österholm P., Nyberg M. E. & Gustafsson J. P. (2012), Metal speciation in rivers affected by enhanced soil erosion and acidity, *Applied Geochemistry*, **27**, Issue 4, pages 906–916

Ormerod S. J., Poole P., McCahon C. P., Weatherley N. S., Pascoe D. & Edwards R. W. (1987), Short-term experimental acidification of a Welsh stream: comparing the biological effects of hydrogen ions and aluminium, *Freshwater Biology*, **17**, pages 341–356

Palko J. (1994), *Acid sulphate soils and their agricultural and environmental problems in Finland*. Ph.D. thesis, University of Oulu, Finland. 58 pages

Palko J. & Yli-Halla M. (1990), Solubility of Al, Cr, Cu and Zn in Soils from a Finnish Acid Sulphate Soil Area., *Aceta Agric. Scand*., **40**, Issue 2, pages 117–122

Parnell J. & Waldren S. (1996), Detrended Correspondence Analysis in the Ordination of Data for Phenetics and Cladistics, *Taxon*, **45**, Issue 1, pages 71–84

Peuranen S., Vuorinen P. J. & Hollander A. (1994), The effects of iron, humic acids and low pH on the gills and physiology of brown trout (S. trutta). *Ann. Zool. Fennici,* **31**, pages 389–396

Raddum G. G. & Fjellheim A. (1987), Effects of pH and aluminium on mortality, drift and moulting of the mayfly Beatis rhodani, *Annis Soc. r. Zool. Belg*., **117**, pages 77–87

Rainbow P. S., Hildrew A. G., Smith B. D., Geatches T. & Luoma S. N. (2012), Caddisflies as biomonitors identifying thresholds of toxic metal bioavailability that affect stream benthos, *Environmental pollution*, **166**, pages 196–207

Rickard D. (2012), *Sulfidic sediments and sedimentary rocks, Developments in Sedimentology*, **65**, Elsevier

Roos M. & Åström M. (2005), Hydrochemistry of rivers in an acid sulphate soil hotspot area in western Finland, *Agricultural and Food Science*, **14**, Issue 1, pages 24–33

Rosemond A. D., Reice S. R., Elwood J. W. & Mulholland P. J. (1992), The effects of stream acidity on benthic invertebrate communities in the south-eastern United States, *Freshwater Biology*, **27**, pages 193–209

Ruuth H. J. (2017*), Kadmiumaltistuksen havaitseminen Hydropsyche pellucidula vesiperhostoukilla (Trichoptera: Hydropsychidae) hyperspektrikuvantamisen ja kidusvaurioiden avulla*, Jyväskylän Yliopisto, Matemaattis-luonnontieteellinen tiedekunta, Bio- ja ympäristötieteiden laitos Akvaattiset tieteet, Master Of Science Thesis (in Finnish), 32 pages

Saarinen T., Vuori K-M., Alasaarela E. & Kløve B. (2010), Long-term trends and variation of acidity, COD_{Mn} and clour in coastal rivers of Western Finland in relation to climate and hydrology, *Science of the Total Environment*, **408,** pages 5019–5027

Salmelin J., Vuori K-M. &Hämäläinen H. (2015), Inconsistency in the analysis of morphological deformities in chironomidae (Insecta: Diptera) larvae, *Environmental Toxicology and Chemistry*, **34**, Issue 8, pages 1891–1898

Soivio A., Myllynen K., Pakkala J. & Jokela S. (1998), Smolting of the brown trout (Salmo trutta L.) in Lestijoki water, *Boreal environment research*, **3**, pages 387–393

Sundermann A., Gerhardt M., Kappes H. & Haase P. (2013), Stressor prioritisation in riverine ecosystems: Which environmental factors shape benthic invertebrate assemblage metrics?, *Ecological Indicators*, **27**, pages 83–96

Sundermann A., Pauls S. U., Clarke R. T. & Haase P. (2008), Within-stream variability of benthic invertebrate samples and EU Water Framework Directive assessment results, Fundamental and Applied Limnology, *Archiv für Hydrobiologie*, **173**, Issue 1, pages 21–34

Sundermann A., Stoll S. & Haase P. (2011), River restoration success depends on the species pool of the immediate surroundings, *Ecological Applications*, **21**, Issue 6, pages 1962–1971

Sundström R., Åström M. & Österholm P. (2002), Comparison of the Metal Content in Acid Sulfate Soil Runoff and Industrial Effluents in Finland., *Environ. Sci. Technol*., **36**, Issue 20, pages 4269–4272

Toivonen J. & Österholm P. (2011), Characterization of acid sulfate soils and assessing their impact on a humic boreal lake, *Journal of Geochemical Exploration*, **110**, Issue 2, pages 107 – 117

Toivonen J., Fröjdö S. & Österholm P. (2019), Impact of acid sulfate soil catchments on water quality in a lake in western Finland; trends and total metal load, *Boreal Environment Research*, **24**, pages 79–99

Tolkamp H. H. & Highler L. W. G. (1983), Hydropsychidae as Bio-Indicators, *Environmental Monitoring and Assessment*, **3**, Issue 3-4, pages 331–341

Tolkkinen M., Mykrä H., Annala M., Markkola A. M., Vuori K-M. & Muotka T. (2015), Multi-stressor impacts on fungal diversity and ecosystem functions in streams: natural vs. anthropogenic stress, *Ecology*, **96**, Issue 3, pages 672–683

Tolonen M. (2002), Perhonjoen keski- ja alaosan vedenlaadun tarkkailu vuosina 1990–2000, Länsi-Suomen Ympäristökeskuksen moniste, **73**, Tutkimusosasto, (Report in Finnish)

Wallace B. J. (1975), Food Partitioning in Net-spinning Trichoptera Larvae: Hydropsyche venularis, Cheumatopsyche etrona, and Macronema zebratum (Hydropsychidae*), Annals of the Entomological Society of America*, **68**, Issue 3, pages 463–472

Willoughby L. G. (1988), The Ecology of *Baetis muticus* and *Baetis rhodani* (Insecta, Ephemeroptera), with Special Emphasis on Acid Water Backgrounds, *Hydrobiology*, **73**, Issue 3, pages 259–273

Virtanen S., Puustinen M. & Yli-Halla M. (2017), Oxidation of iron sulfides in subsoils of cultivated boreal acid sulfate soil fields – based on soil redox potential and pH measurements, *Geoderma*, **308**, pages 252–259

Wu X., Wong Z. L., Sten P., Engblom S., Österholm P. & Dopson M. (2013), Microbial community potentially responsible for acid and metal release from an Ostrobothnian acid sulfate soil*, FEMS Microbiology Ecology*, **84**, Issue 3, pages 555–563

Vuori K-M. & Kukkonen J. (1996), Metal conentration in Hydropsyche pellucidula larvae (Trichoptera, Hydropsychidae) in relation to the anal papillae abnormalities and age of exocuticle, *Wat. Res*., **30**, Issue 10, pages 2265–2272

Vuori K-M. & Parkko M. (1996), Assessing pollution of the river Kymijoki via hydropsychid caddisflies: population age structure, microdistribution and gill abnormalities in the Cheumatopsyche lepida and Hydropsyche pellucidula larvae, *Arc. Hydrobiol*., **136**, pages 171–190

Vuori K-M. (1993), Influence of Water Quality and Feeding Habits on the Whole-Body Metal Concentrations in Lotic Trichopteran Larvae, *Limnologica*, **23**, Issue 4, pages 301–308

Vuori K-M. (1996), Acid-induced acute toxicity of aluminum to three species of filter feeding caddis larvae (Trichoptera, Arctopsychidae and Hydropsychidae), *Freshwater Biology*, **35**, pages 179–188

Vuori K-M. (2002), Vesisammal- ja vesiperhosmenetelmät jokivesistöjen haitallisten aineiden riskinarvioinnissa ja seurannassa, Länsi-Suomen Ympäristökeskus, 571, Kokkola, Finland (in Finnish)

Vuorinen M., Vuorinen P. J., Hoikka J. & Peuranen S. (1993), Lethal and sublethal threshold values of aluminium and acidity to pike (Esox Lucius), whitefish (Coregonus lavaretus pallasi), pike perch (Stizostedion lucioperca) and roach (Rutilus rutilus) yolk-sac fry., *The Science of the Total Environment*, **134**, Issue 2, pages 953–967

Yli-Halla M., Puustinen M. & Koskiaho J. (1999), Area of cultivated acid sulfate soils in Finland, *Soil Use and Management*, **15**, pages 62–67

Åström M. & Björklund A. (1995), Impact of acid sulfate soils on stream water geochemistry in western Finland, *Journal of Geochemical Exploration*, **55**, Issues 1– 3, pages 163–170

Åström M. & Björklund A. (1996), Hydrogeochemistry of a stream draining sulfide-bearing postglacial sediments in Finland, *Water, Air, and Soil Pollution*, **89**, Issue 3–4, pages 233–246

Åström M. & Björklund A. (1997), Geochemistry and acidity of sulphide-bearing postglacial sediments of western Finland., *Environmental Geochemistry and Health*, **19**, Issue 4, pages 155–164

Åström M. & Åström J. (1997), Geochemistry of stream water in a catchment in Finland affected by sulphidic fine sediments, *Applied Geochemistry*, **12**, Issue 5, pages 593–605

Åström M. (1998), Partitioning of transition metals in oxidised and reduced zones of sulphide-bearing fine-grained sediments., *Applied Geochemistry*, **13**, Issue 5, pages 607–617

Åström M. (2001), The effects of acid soil leaching on trace element abundance in a medium-sized stream, W. Finland, *Applied Geochemistry*, **16**, pages 387–396

Åström M., Sundström R. & Holmberg M. (2005), pH of streams in western Finland - a perspective from the Middle Ages into the mid 21st century, *Agricultural and Food Science*, **14**, Issue 1, pages 5–13

Österholm P. (2005), *Previous, Current and Future Leaching of Sulphur and Metals from Acid Sulphate soils in W. Finland*, PhD thesis, Åbo Akademi, Department of Geology and Mineralogy, Turku, Finland

Österholm P. & Åström M. (2002), Spatial trends ans losses of major and trace elements in agricultural acid sulphate soils distributed in the artificially drained Rintala area, W. Finland. *Applied Geochemistry*. **17**, pages 1209–1218