CLADOCERA AS SENTINELS OF AQUATIC MINE POLLUTION

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ACADEMIC DISSERTATION

To be presented for public examination, with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, in Auditorium 2, Info Centre Corona, (Viikinkaari 11), on 5th of April 2019, at 12 noon.

Helsinki, 2019
The mining industry has a critical role in modern societies. However, the environmental harm induced by mining activities may be drastic. One of the most important aspects of mining pollution is water contamination due to accidental or controlled waste water release, or due to harmful leachate originating from waste areas.

Whereas the water quality in receiving water systems is usually monitored, the ecological impacts of mine waters are assessed more rarely. One of the challenges when ecological impacts are studied is the lack of long-term data regarding the pre-mining conditions.

Palaeolimnological methods allow the reconstruction of historical conditions of water bodies. Sedimentary geochemistry and subfossil remains of biological communities (e.g. cladoceran crustacea, diatom algae) can be used to assess the natural pre-disturbance variability, the impact of the disturbance and the post-disturbance dynamics.

In this thesis, mining pollution is assessed within three projects, conducted on two boreal lakes in Finland. In Lake Kirkkojärvi, the impact of historical mining activity, namely, the direct release of mineral tailings was studied from a sediment core covering also the pre-mining environmental conditions. The impacts of the mine pollutants were reflected in cladoceran communities as declining species richness and diversity. The lake Kirkkojärvi bay was studied by using a palaeolimnological top-bottom approach. The bay has been receiving acid mine drainage for nearly 50 years. However, the cladoceran community change in the bay does not reflect any changes in acidity or metal pollution. In Lake Kivijärvi the Na₂SO₄- and metal-contaminated mine water has had a clear impact on cladoceran and diatom communities as both groups exhibit changes due to mining pollution. Based on the results, the most important contaminants in the studied systems are saline effluents and mineral tailings.

Cladocera hold great potential in palaeolimnological mining pollution studies due to their sensitivity to changes in the water quality and due to their central location in food-webs. However, more research is still needed: Most importantly, some taxonomic issues must be clarified and in-situ toxicity studies should be conducted in order to find the most sensitive indicator species for different types of pollution.
The results of this work present important information regarding pollution impact assessment. In particular, this study highlights the utility of Cladocera as early warning indicators, while supporting the use of multiple sediment proxies in palaeolimnological pollution research in order to provide information about the timing, direction, and magnitude of ecosystem impacts caused by pollution events. This is of essence for environmental management as globally the exploitation of poor-grade large mine deposits will drastically increase in the near future. Only by providing such crucial information on the short- and long-term impacts of mining on aquatic ecosystems and their resilience, a technologically, socially and environmentally sustainable management of mines can be guaranteed in the future.
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:


III Leppänen, J., Weckström, J., Korhola, A. 2017: Multiple mining impacts induce widespread changes in ecosystem dynamics in a boreal lake. Scientific Reports 7(1) DOI: 10.1038/s41598-017-11421-8
CONTRIBUTIONS TO THE PUBLICATIONS

Abbreviations for the names included in the articles. JL - Jaakko Leppänen; JW - Jan Weckström; AK - Atte Korhola


The original idea and initial study plan was developed by JL. JL and JW collected the samples and conducted the numerical analyses. JL was responsible for cladoceran analysis and for writing the draft of the manuscript. All authors contributed to the manuscript.


The original idea and initial study plan was developed by JL. JL and JW collected the samples. Cladoceran and numerical analyses were conducted by JL. JL was responsible for writing the draft of the manuscript. All authors contributed to the manuscript.

III Leppänen, J., Weckström, J., Korhola, A. 2017: Multiple mining impacts induce widespread changes in ecosystem dynamics in a boreal lake. Scientific Reports 7(1) DOI: 10.1038/s41598-017-11421-8

The study was planned by JL, JW, and AK. JL collected the samples. JL analysed the cladoceran and JW the diatom communities. JL and JW conducted the numerical analysis. JL was responsible for writing the draft of the manuscript. All authors contributed to the manuscript.
# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AMD</td>
<td>Acid mine drainage</td>
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<td>ARD</td>
<td>Acid rock drainage</td>
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<td>e.g.</td>
<td>exempli gratia</td>
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<td>i.e.</td>
<td>id est</td>
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<td>s.l.</td>
<td>sensu lato</td>
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1 INTRODUCTION

Humans have modified freshwater ecosystems to the point that their biodiversity is reduced and the ecosystem services they provide are compromised (Vörösmarty et al. 2010). Water quality is directly linked to water availability as declining water quality hinders the use of water. Thus, freshwater scarcity is a rapidly growing global concern (Kummu et al. 2016) and currently 82 % of the global population rely on freshwater sources that are highly threatened (Green et al. 2015).

Mining is one of the most important point source pollutants of freshwaters (Schwarzenbach et al. 2010). This is due to the high magnitude of mining activities which, in turn, are fuelled by the high demand of materials in modern societies. The mining-based effluents may contain high concentrations of harmful components (Cravotta 2008; Nordstrom 2011), thus posing a risk to the receiving waters. Although the impact of a single mine on the environment is generally a local problem, mining pollution adds to some of the most concerning environmental issues such as acidification (Weiss et al. 2018), toxic pollution (MEA 2005) and salinization (Cañedo-Argüelles et al. 2013; Kaushal et al. 2018) of freshwaters.

Monitoring programs are implemented in many countries to assess the impact of mining activities on the environment. However, these time-series only rarely cover the time before the mining-induced disturbance, which would enable the assessment of the natural variability of the polluted system. Moreover, measurements of water chemistry, which are usually the most important component in pollution monitoring, are alone not sufficient enough to provide a holistic overview of the ecological impacts of pollution. A comprehensive assessment of the ecological impacts of mine water pollution could be provided by adding e.g. bio-indicator groups (Bondaruk et al. 2015), and by using palaeolimnological methods also the temporal aspect could be addressed (palaeo-ecotoxicological framework; Korosi et al. 2017).

In this work, I investigate the impacts of mining pollution on cladoceran communities in two boreal lakes in Finland. The aim is to assess the history of cladoceran communities including the periods before, during and after the
mining (Paper I), to identify the impacts of chronic low volume acid mine drainage (Paper II) and to study the ecological impacts of saline effluents originating from a modern polymetal mine (Paper III).

1.1 MINING AND THE ENVIRONMENT

The mining sector is of critical importance to modern societies, but it is regarded as a high risk activity in terms of its environmental impacts. As the known high quality deposits are becoming increasingly depleted, the mining industry is investing to exploit low grade ores (Watling 2015). Subsequently, even though the number of mines has decreased during the past decades, the volume of extracted minerals has actually doubled during the past 30 years (Reichl et al. 2016). One of the most important environmental impacts of mining is linked to mine waste. Currently, thousands of millions of tons of mine waste is being produced annually and the quantity of mine waste is increasing exponentially due to the establishment of new mines exploiting low grade ores (Hudson-Edwards & Dold 2015). Many types of waste are generated in mines. The most important types of mine wastes are waste rock, tailings and mine water, all of which exhibit potential for environmental damage, depending on rock chemistry, type of processing reagents and waste management practices. The most common methods for mine waste disposal are the discharging of slurry tailings into tailings ponds, backfilling the waste into underground mine tunnels or open pits, and discharging the tailings subsurface into a lake or the sea. Waste rock is usually considerably less harmful compared to tailings and waste rock can occasionally be used as construction material for roads or tailings dams (European Commission 2009). Large ponds and dam systems always carry a risk of leaks and failures, which have at several occasions been reported (Feasby et al. 1999; Soldan et al. 2001; Escobar 2015). In addition to permanently stored waste, mines need to take care of storm-waters and process-waters, which may contain harmful constituents. In general, the mine waters are treated for e.g. acidity and harmful elements and purified water is released into the environment. In regions, where precipitation exceeds evaporation, the volume of mine water may become a critical issue if the planned water treatment capacity is insufficient.
1.2 MINE WATER CHARACTERISTICS

Mine waters originate from uncontrolled releases (e.g. leakage or overropping of ponds, unintercepted seepage or leaching from dry heaps), controlled release of process waters, dewatering waters and site runoff waters. Mining pollution is almost always a multi-stressor problem due to the presence of multiple potentially harmful constituents in the mine water. Mine waters may exhibit a wide variability of elemental concentrations (Cravotta 2008) and of other characteristics such as acidity, salinity, radioactivity and corrosivity (Nordstrom 2011). In some cases, the concentrations of toxic substances are high (e.g. 4.25 mg/L U; Ferrari et al. 2009, 1.88 mg/L Cu; Parviainen 2009, 850 mg/L As; Druschel et al. 2004), however in other cases mine waters can have very low concentrations of harmful constituents, thus posing no significant threat to the environment (Banks et al. 1997).

Probably the best known type for mine waste water is acidic leachate, resulting from the oxidation of sulphide bearing minerals (Dold 2014), known as the acid mine drainage (AMD) or acid rock drainage (ARD). AMD usually occurs at the waste storage areas containing tailings and/or waste rock. The AMD begins once the new waste material is no longer deposited and the material is exposed to air and precipitation. Sulphide oxidation results in acid-producing reactions, which subsequently dissolves heavy metals into the solution (e.g. Akcil and Koldas 2006). The phenomenon is notorious for its longevity (e.g. Younger 1997) and is thus an environmental problem, which clearly exceeds the operational time of the mine itself.

1.3 ECOLOGICAL IMPACTS OF MINE WATERS

The ecological impacts of mine waters have been acknowledged for centuries. Already G. Agricola in 1556 (Hoover & Hoover 1950) exemplified the worries caused by mining pollution nearly half a millennia ago: “Further when the ores are worked, the water which has been used poisons the brooks and streams, and either destroys the fish or drives them away. . . . Thus, it is said, it is clear to all that there is greater detriment from mining than the value of the metals which mining produces”

The impacts of mine pollution are usually most distinct in small-volume waters (e.g. narrow streams), which receive large amounts of mine drainage,
but where dilution is limited. Once the mine water reaches a larger body of water, the drainage is diluted and a pollution gradient is established. A pollution gradient with corresponding differences in species assemblages is commonly reported (Rasmussen & Sand-Jensen 1979; Foster 1982; Belyaeva & Deneke 2007; Kihlman & Kauppila 2009; Bier et al. 2015). Because mine waters commonly contain more than one potentially harmful component, the strength of the final impact depends on the mixture effect of the constituents (Biesinger et al. 1986; Norwood et al. 2003; Traudt et al. 2017). For example, overadditive effects (the mixture is more toxic that is expected based on the toxicity of the individual components) have been detected for various metals (e.g. Cu, Pb, Zn for cladoceran crustacea; Cooper et al. 2009) whereas the hardness-causing cations (e.g. Ca$^{2+}$ and Mg$^{2+}$) may decrease the toxicity of metals in mine water (Yim et al. 2006). In general, the combination of acidic pH and elevated metal concentrations efficiently reduces species richness (Hargreaves et al. 1975; Wollmann et al. 2000) and the number of taxa is extremely low in heavily impacted sites (e.g. Salonen et al. 2006; Hogsen & Harding 2012). In the most affected sites, the biota may be composed of only few specialized species, such as the green alga *Chlamydomonas acidophilia* and the protist *Euglena mutabilis* (Kelly 1988). In addition to acidic mine waters, increased salinity of freshwaters is a common consequence of mining pollution. The saline mine drainage is often considered as a less serious problem than the AMD, which can be seen as less consistent regulations regarding mitigation or treatment of saline mine waters (Opitz & Timms 2016). However, already low- salinity effluents may cause negative impacts on freshwater biota (Schallenberg et al. 2003) and the environmental damage due to saline mine waters has been acknowledged (Zgór ska et al. 2016). In general, species with low internal ionic concentrations and poor osmoregulatory capacity are eliminated first and followed by more salinity-tolerant species (e.g. James et al. 2003). Moreover, in cases of high magnitude salinisation the permanent stratification (or meromixis) (Fig 1), induced by saline mine water (e.g. Schultze et al. 2017), may efficiently terminate the oxygen and nutrient circulation and factually render the deep bottoms inhabitable (e.g. Boehrer & Schultze 2008).
1.4 CLADOCERA

Cladocera form a group of mostly microscopic (size usually 0.2 mm – 1.5 mm) zooplankton crustacea, which inhabit nearly all types of aquatic habitats (Błędzki & Rybak 2016). The body of most cladoceran species is oval – shaped, and it is covered by chitinous shell valves and a headshield (Smirnov 2017). The group consists of planktonic, benthic and plant-associated taxa, and while most of the species feed on algae, detritus and bacteria, few species are predators (Błędzki & Rybak 2016). In general, planktonic species are filter feeders, whereas the littoral species are scrapers (Fryer 1987). Cladocera are somewhat in the middle of the food-web, as they act as a linkage between the primary producers and the secondary consumers. Thus, according to de Bernardi et al. (1987), Cladocera are a key group among freshwater zooplankton and their interactions can be considered of high importance in aquatic food chains.

Four cladoceran orders are currently recognised: Anomopoda, Ctenopoda, Onychopoda and Haplopopoda (Forro et al. 2008) consisting over 700 species (Smirnov 2017), but the group is under constant revision (e.g. Korovchinsky 1997; Kotov et al. 2009; Kotov 2015). The taxonomy issues are closely related to cladoceran biogeography, and species, which were earlier claimed to be cosmopolitan are actually groups or complexes of species (Frey 1987). Further, only 45-50 % of the currently known species are well described, whereas the status of other species is more or less unsure. Many of those may
represent cryptic complexes (Korovchinsky 1996). These taxonomical issues are likely to increase the number of cladoceran species in the future, as new species are being found and described. For example, a new family, Dumontiidae, was recently described (Santos-Flores & Dodson 2003). Advances in DNA technology are likely to further alter cladoceran taxonomy (e.g. Bekker et al. 2016). Thus, the statement by Frey (1987) “But anyone who has worked with Cladocera knows the frustration of not being certain that the name he is using for a taxon is the correct one” still remains.

Cladocera are usually highly abundant during the growing season as the community reproduces rapidly in favourable conditions. Then, most cladoceran species reproduce asexually by cyclical parthenogenesis and, in northern climates (e.g. in Finland), switch to sexual reproduction once the environmental conditions become less favourable (e.g. low temperatures and food scarcity; Frey 1982). The sexually produced progeny is a diapausing egg (resting egg). Once conditions are improved (e.g. spring) the hatching of the resting egg is triggered by light and temperature signals (Vandekerkhove et al. 2005). The hatched individuals are always females, which begin the parthenogenetic reproduction and the cycle starts over again. The resting egg is a mechanism to re-establish the community after a stress. In addition, this mechanism generates new genotypes and plays also a part in species dispersal (Frey 1982). Among some species (anomopods), the resting egg is encased in a shell (ephippium), which can stay viable after extended periods of time. They can withstand desiccation and even survive the passing through a bird’s digestive system (Figuerola & Green 2002).

In general, the cladoceran community structure is controlled by lake size and productivity (e.g. Dodson 1991; Dodson 1992). According to Angermeier and Schlosser (1989), there are three theories, which explain the linkage between ecosystem size and species richness. Larger lakes tend to contain higher number of niches, have lower extinction rates and higher immigration rates. The importance of productivity (and related characteristics) is well demonstrated in studies considering long time-series in lake productivity (Davidson et al. 2011) or “snap-shot” studies, where the sampling sites form an eco-climatic gradient (e.g. spanning from boreal forest to treeless higher altitude tundra; Leppänen et al. 2017). Other factors controlling species assemblages include, but are not limited to, predator regime (Milardi et al.
2016), pH (Zawisza et al. 2016) and waterbody lifetime (in temporary waterbodies; Evdokimov & Ermokhin 2009).

1.4.1 THE USE OF CLADOCERA IN RESEARCH

Cladocera have been studied for centuries. According to Korovchinsky (1997) the first reported morphological and taxonomic studies date to the latter half of the 17th century. The applicability of Cladocera as test organisms was considered at the end of the 19th century by Metchnikoff (cited in Lampert 2011). One of the first studies, where cladoceran population dynamics were linked to environmental factors, was conducted by Berg (1934). Since then, Cladocera have established an important status in environmental research.

Cladocera (especially the genus *Daphnia*) are frequently used organisms in ecotoxicological studies (e.g. Sarma & Nandini 2006; Martins et al. 2007; Persoone et al. 2009). In addition, *Daphnia* has been used for example in studies dealing with the grazing effects on phytoplankton, the phenomenon of diel vertical migration of zooplankton, and in studies considering cyclomorphosis and inducible defenses (Lampert 2011). Moreover, due to their central location in food-webs, Cladocera have been used in food-web studies, where for example the presence of fish is controlled resulting in trophic cascades (e.g. Carpenter et al. 1987; Declerck et al. 1997). This is of high importance in lake restoration projects.

Cladocera are excellent indicators of environmental change (Eggermont & Martens 2011) responding both via community shifts (Korhola et al. 2000; Nykänen et al. 2006; Nevalainen & Luoto 2013) and morphological changes (e.g. Korosi et al. 2013; Nevalainen & Luoto 2013). Further, Cladocera are more sensitive towards water pollution than many other aquatic organisms (e.g. metals; Brix et al., 2001; Von Der Ohe and Liess, 2004). In addition to the summary by Korhola and Rautio (2001), linkages between environmental variables and the cladoceran community structure have recently been studied in many regions, e.g. in northern Finland (Leppänen et al. 2017), in Ugandan crater lakes (Rumes et al. 2011), in Central America (Wojewódka et al. 2016), and in dystrophic lakes in central Europe (Zawisza et al. 2016). Thus, the factors controlling the cladoceran species composition are relatively well known.
1.4.2 CLADOCERA IN MINING POLLUTION STUDIES

Cladocera are extensively used as in-vitro test organisms in mine water monitoring. In Canada, for example, mining companies are obliged to conduct *Daphnia* toxicity tests to assess the quality of mine water (Canadian Government 2002). In addition to monitoring programs, cladoceran laboratory studies have been conducted to identify the cause of toxicity in saline mine water environments (Van Dam et al. 2014), to discriminate between the pH and heavy metal toxicities in acid mine drainage (Lopes et al. 1999), to evaluate the importance of sediment contamination (Pereira et al. 2000), and to assess the toxicity of different types of mine drainage (Lee et al. 2015). In addition to laboratory studies, Cladocera have been studied at many mining pollution sites globally (review by Leppänen 2018 and references therein). However, only a portion of the studies include an adequate temporal dimension which covers pre-mining conditions (e.g. Bradbury & Megard 1972; Coard et al. 1983; Melville 1995; Kerfoot et al. 1999; Kurek et al. 2012; Doig et al. 2015; Chen et al. 2016; Sienkiewicz & Gasiorowski 2016; Winegardner et al. 2017) and complete species inventories are only rarely published. Cladoceran studies have concentrated on impacts of typical mine water characteristics, i.e. acidity, heavy metals and elevated salinity (Leppänen 2018).

The mechanism behind harmful impacts of acidity is related to the interference with cladoceran sodium intake (e.g. Vangenechten et al. 1989), whereas heavy metals affect the respiration and Mg homeostasis (Ni; Pane et al. 2003) and inhibit the active sodium uptake (Ag; Bianchini & Wood 2003) of *Daphnia magna*. Elevated salinity has in general negative impacts on freshwater cladoceran populations (Elphick et al. 2011; Van Dam et al. 2014). Because salinity tolerance varies among different species (Aladin & Potts 1995), changes in salinity also induce shifts in species compositions (Aladin 1991). In many mining pollution studies, *Bosmina longirostris* and *Chydorus sphaericus* are reported to tolerate mine water-impacted conditions (Leppänen 2018). However, the taxonomy and biogeography of both species are problematic and currently under revision (*Bosmina*; De Melo & Hebert 1994; Kotov et al. 2009, *Chydorus*; Belyaeva & Taylor 2009; Kotov et al. 2016). Thus, any global generalizations regarding these species should be met with caution.
1.5 DIATOMS

Diatoms (Bacillariophyta) are a group of microscopic siliceous single-celled algae which are encountered in nearly all aquatic systems and in moist terrestrial habitats (e.g. Round 1990). The number of currently recognized species is ca. 12000 but the total number of species is likely much higher, perhaps as high as 100000 species (Mann & Vanormelingen 2013). Similarly to Cladocera, the improved DNA techniques have greatly contributed to the increased knowledge of diatom diversity (e.g. Malviya et al. 2016).

The first reported observation of diatoms was made by an unknown Englishman in the beginning of 18th century (Anonymous 1703). Today, diatom community structure is known to reflect aquatic environmental conditions, and they are frequently used as proxies for assessing changes in variables such as pH (e.g. ter Braak & Van Dam 1989; Weckstöm et al. 1997), salinity (Fritz et al. 1991) and phosphorus (e.g. Anderson & Rippey 1994; Miettinen et al. 2005). The group has been used extensively in ecotoxicology (e.g. Debenest et al. 2013) and mining pollution research (e.g. Archibald & Taylor 2007; Kihlman & Kauppila 2010; Tuovinen et al. 2012; Sienkiewicz & Gasiorowski 2016; Sienkiewicz & Gasiorowski 2018).

1.6 LAKE SEDIMENTS AS POLLUTION DIARIES

Lake sediments can be regarded as environmental libraries, containing a great amount of information of the environmental history of the lake and its catchment area. Palaeolimnology can be used to reveal past limnological and environmental conditions of aquatic ecosystems. The palaeolimnological approach is based on the fact that sedimentation areas of waterbodies act as sediment traps, where new material is continuously accumulated above the older sediment material (e.g. Frey 1988; Smol 2008). The vertical sediment record thus forms a temporally structured archive of environmental history. The vertical sediment series are usually retrieved using a variety of tube corers, which preserve the vertical record intact allowing chronological analysis. The sediment samples can be dated (e.g. using radioisotopic methods such as $^{210}$Pb, $^{137}$C; Appleby 2001, $^{14}$C; Björck & Wohlfarth 2001), and the subsampled sediment sections linked to the corresponding dates. Occasionally, the sediment section can be annually laminated (Lamoureux 2001). Temporal chronology is essential in order to be able to assess the speed
and magnitude of possible changes. Although the sediment cores are usually analysed sequentially, sometimes it is more appropriate to apply the so called top-bottom (before-after) method (e.g. Michelutti et al. 2001; Weckström et al. 2003; Korosi and Smol 2012), where the sediment is analysed from two samples: The bottom sample represents a time period before a point of interest (e.g. time before the pollution began) and the top sample records the later date (usually contemporary situation recorded in the samples collected from the sediment surface).

By using the palaeolimnological approach it is possible to reconstruct the characteristics of environmental pollution and its ecological impacts (e.g. Smol 2008). Lake sediments, for example, provide a diary of the sedimentary concentrations of harmful substances (e.g. Blais et al. 2015). However, the interpretation of the elemental sequence and comparisons between their sedimentary and aquatic concentrations are not always straightforward (Outridge & Wang 2015). This is because redox conditions, pH, and availability of oxygen are known to affect transformation and migration of elements in sediments (Carignan & Nriagu 1985; Belzile & Morris 1995). Another powerful and frequently used palaeolimnological tool are the sedimentary subfossil remains of a number of organisms (e.g. chironomid larvae, ostracods, diatoms, and cladocerans). Based on their ecological preferences, these palaeobiological indicators can be used to assess the impacts of pollution in biological communities (Smol 2008). The applicability of cladoceran crustaceans in palaeolimnology has been acknowledged for decades (Frey 1960). However, whereas some groups e.g. Chydoridae and Bosminidae are known to preserve very well in sediments, some other species show only limited preservability (Korhola & Rautio 2001) and others (e.g. *Daphnia cucullata*) may show temporal variability in preservation even in a single lake (Leppänen & Weckström 2016), thus limiting the applicability of some species. Moreover, due to different habitat preferences among taxa and subsequent differences in the deposition areas between littoral and pelagic species, the similarity between living cladoceran community and the community reconstructed from sediment samples is not always identical (e.g. Kattel et al. 2006). Diatoms have been used in palaeolimnological studies since the mid-19th century (Bradbury 1988) and the group is probably the most used indicator group in palaeoenvironmental reconstructions due to the generally excellent preservation of siliceous cell walls and well-established environmental preferences of a number of taxa (Moser et al. 1996).
1.7 AIMS OF THE THESIS

The research hypotheses and aims were:

Hypothesis 1)

Cladoceran communities reflect the different phases of mining, such as tailings dumping and post-mining acid mine drainage in receiving lake systems.

Aim and execution
To assess whether and how the cladoceran community was affected by mining activities and the post mining AMD, we analysed the cladoceran species composition dynamics from the past decades in a lake where the history of mining activity is relatively well recorded (Paper I).

Hypothesis 2)

The impact of chronic but low volume AMD may be visible in cladoceran communities.

Aim and execution
To study whether the cladoceran assemblages are affected by chronic AMD and whether the community structure varies along the pollution gradient, we compared the contemporary and pre-mining communities at multiple sampling sites in the embayment of Lake Kirkkojärvi (Paper II).

Hypothesis 3)

The Na₂SO₄ - and metal-contaminated biomining effluent, originating from controlled waste water release and a dam failure, may induce rapid changes in lake environments and these damages can be assessed using palaeolimnological methods.

Aim and execution
To determine how the lake biota has been impacted by modern biomining effluent we combined limnological times-series data, land use history, and palaeolimnological data from Lake Kivijärvi to evaluate the ecological
impact of the uncontrolled mine water discharges from the large-scale but low-grade open pit Talvivaara mine on the lake (Paper III).
2. MATERIALS AND METHODS

2.1 STUDY SITES

Two boreal lakes were selected to study the impacts of mine pollution on aquatic ecosystems. The lakes are located in southern (Lake Kirkkojärvi) and central (Lake Kivijärvi) Finland (Fig 2; Table 1). The study sites were selected due to available supporting information regarding the land use histories and mining activities, thus allowing the detailed temporal analysis of environmental changes and possible forcing factors.

Lake Kirkkojärvi is a small (0.7 km²) boreal lake located in Viljakkala, southern Finland. The lake is connected to considerably larger water bodies Lake Viljakkalanselkä (5 km²) and Lake Kyrösjärvi (92 km²). Lake Kirkkojärvi can be regarded as a sub-basin of Lake Viljakkalanselkä and it is considered mesotrophic with nearly neutral pH (Kihlman & Kauppila 2010). The catchment area is characterized by agricultural fields and forests. The water level of Lake Kirkkojärvi has been lowered several times during the nineteenth century (Vänni 1928) resulting in a ca. 3 m drop in water level. Due to these changes, the SW channel between Lake Kirkkojärvi and Lake Kyrösjärvi has disappeared thus affecting the water circulation properties of Lake Kirkkojärvi (Paper I; Fig 1). The region has a long history in mining activities and industrial-scale copper and gold mining began in the nearby Haveri deposit in 1940 (Kaskimies & Sinisalo 1973). The mine waste from the processing facility was channelled directly into the lake during the mining operations between 1940 and 1960. The waste area was dammed in the late 1950s (Pöyry 2015), but the tailings dam failure during the 1960s opened a channel for drainage waters originating from the waste area into the adjacent lake embayment (Papers I and II). The drainage water is acidic (pH 3.4 and exhibits high soluble concentrations of multiple metals, e.g. Cu 1670 μg/L, Ni 692 μg/L, Al 24300 μg/L; Parviainen 2009). Previous palaeolimnological studies in Lake Kirkkojärvi have reported distinct changes in diatom and protists assemblages (Kihlman & Kauppila 2010).

Lake Kivijärvi is a small (1.8 km²) boreal lake located in Sotkamo, central Finland. The catchment vegetation is dominated by forests and peatlands.
Currently, the human population in the lake catchment is less than 20 inhabitants. The strongest (pre-mining) human impact in the catchment area occurred in the 1960s and 1970s as intensive forestry (ditching) operations (Leppänen & Weckström 2016). The Talvivaara Terrafame polymetal mine is located ca. of 5 km NE of the lake. Lake Kivijärvi is connected to the Talvivaara Terrafame mine via a chain of small lakes and a river (River Lumijoki). The waste waters from the mine are released to the River Lumijoki after treatment (Finnish Safety Investigation Authority 2014). However, the mine has struggled with the handling of the waste water since the beginning of the mining operations, which started in 2008. Since then, the receiving water systems have been impacted by metal-contaminated and saline (Na₂SO₄) waste waters. In addition, the mine has experienced two major dam failures in 2012 and 2013 resulting in the release of over 240 000 m³ of process waste water into the environment (Paper III). The environmental impacts of the Talvivaara Terrafame mine have been widely discussed in Finland during the recent years (Sairinen et al. 2017), however, studies about the ecological impact of mine pollution on aquatic ecosystems are sparse.
Figure 2. Study site locations. The red dots indicate sampling site locations (I,II Lake Kirkkojärvi; III Lake Kivijärvi). The map data (Open Database Licence 1.0) was retrieved from OpenStreetMap Foundation GIS database (http://www.geofabrik.de) and edited with ArcMap 10.3.1 and CorelDraw 2017.

Table 1. General characteristics of the study sites. Both temperature and precipitation represent mean annual values. For references, see papers I, II and III.

<table>
<thead>
<tr>
<th>Study site</th>
<th>Lake Kirkkojärvi (Paper I)</th>
<th>Lake Kirkkojärvi bay (Paper II)</th>
<th>Lake Kivijärvi (Paper III)</th>
</tr>
</thead>
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<tr>
<td>N (WGS 84)</td>
<td>61.7148</td>
<td>61.7148</td>
<td>63.9266</td>
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<tr>
<td>E (WGS 84)</td>
<td>23.2677</td>
<td>23.2677</td>
<td>27.9111</td>
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<tr>
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<td>83</td>
<td>165</td>
</tr>
<tr>
<td>Temperature (°C)</td>
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<td>4-5</td>
<td>2</td>
</tr>
<tr>
<td>Precipitation (mm)</td>
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<td>600–650</td>
<td>600</td>
</tr>
<tr>
<td>Catchment</td>
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<td>Agricultural fields, forests</td>
<td>Coniferous forests, peatland</td>
</tr>
<tr>
<td>Lake area (km²)</td>
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<td>0.05</td>
<td>1.8</td>
</tr>
<tr>
<td>Average depth (m)</td>
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<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Maximum depth (m)</td>
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<td>2.7</td>
<td>12</td>
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<tr>
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<td>Mesotrophic</td>
</tr>
<tr>
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<td>Haveri</td>
<td>Haveri</td>
<td>Talvivaara</td>
</tr>
<tr>
<td>Pollutants</td>
<td>Tailings, AMD</td>
<td>AMD</td>
<td>Metals, Na, S</td>
</tr>
</tbody>
</table>
2.2 SAMPLING AND SAMPLE PREPARATION

Lake Kirkkojärvi

The sediment core of Lake Kirkkojärvi was retrieved in May 2016 from the deepest basin (depth of 8.5; Fig 3) using an UWITEC gravity corer (UWITEC, Mondsee, Austria; http://www.uwitec.at/html/frame.html; sediment). The core (44.5 cm in length, 6 cm in diameter) was subsampled at 0.5 cm intervals and stored in plastic bags in a cold room at +4°C prior to analysis.

Lake Kirkkojärvi bay

The data-set consisting of 9 top-bottom samples was retrieved at Lake Kirkkojärvi bay in May 2016 using a Russian peat corer (Jowsey 1966). The sampling depth varied between 1 – 2 m. Each top-bottom pair of samples was sectioned using a metal slate and subsamples were stored in plastic bags in a cold room at +4 °C prior to analysis. The top samples, representing modern conditions, consisted of the topmost 1 cm. The bottom samples (1 cm in thickness), representing the pre-mining period, were retrieved from varying sediment depths between 0.2 – 1.3 m, depending on the location of the pronounced layer of the tailings material. The bottom sample was always retrieved from below the tailings layer. The additional 2 surface sediment samples (1 cm in thickness) were retrieved from reference sites in Lake Kirkkojärvi and Lake Viljakkalanselkä using a HTH Kajak gravity corer (Renberg & Hansson 2008) in July 2016 (Fig 3). The reference sites (R1, R2) differ in land-use activities, however, both are currently not affected by the AMD. In addition, in order to assess the current acidity caused by the mine drainage, pH was measured from a ditch located on the top of the tailings area using a Merck pH—indicator paper (Paper II; Fig 1). Two surface water samples were collected from Lake Kirkkojärvi bay (top bottom sites 1 and 9) and from the water quality reference site located at Lake Viljakkalanselkä (RW) (Fig 3). Water samples were collected in 0.5 litre plastic bottles in July 2016 and stored in a fridge at +4 °C prior to analysis.
Lake Kivijärvi

Four sediment cores (A,B,C,D; with an approximate length of 25 cm) were retrieved from the flat sedimentation basin (depth 9.1 m) of Lake Kivijärvi using a HTH Kajak gravity corer (Renberg & Hansson 2008), and one core (E) using a Limnos sediment sampler (Kansanen et al. 1991) (Fig 4) in March 2015. Cores A,B,C,D were subsampled at 0.25 cm intervals using a plastic slate. Core A was used for cladoceran, diatom and dating analyses, whereas cores B, C and D were combined in order to collect enough material for geochemical analysis. Core E was subsampled at a resolution of 1 cm and used for loss-on-ignition (LOI) analysis. Subsamples were stored in plastic bags at +4 °C prior to analysis. All cores were retrieved undisturbed and were correlated visually based on grey coloured surface encrustation and a distinct colour shift at the depth of 1 cm and more gradual colour transition layer between 4 and 6 cm.
2.2.1 CLADOCERAN ANALYSIS

Cladoceran samples were prepared according to Korhola & Rautio (2001). In brief, sediment samples (~ 1 cm³) were treated in a hot 10% KOH bath for one hour, sieved through a 48 μm sieve with tap water and the saphranine-stained residue was then pipetted onto microscopic slides and mixed with gelatine glycerol for permanent mount slides. Cladoceran remains were identified and counted using an Olympus BX-30 light microscope at a 100-400 x magnification. Species identification and nomenclature was based on Sarmaja-Korjonen & Szeroczynska (2007). In summary, cladocerans were analysed from 32 samples from Lake Kirkkojärvi core (Paper I), from 18 samples from Lake Kirkkojärvi bay and its reference sites (Paper II), and from 16 samples from Lake Kivijärvi core (Paper III).
2.2.2 DIATOM ANALYSIS

Diatoms were analysed from the topmost 4 cm (8 samples) of the Lake Kivijärvi core (Paper III). Diatom samples were prepared using H2O2 digestion and HCl treatment (Weckström et al. 1997). The diatom samples were mounted on Naphrax and the diatom valves were identified and counted along random transects at a x 1000 magnification. Taxonomic identification was mainly based on Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b).

2.2.3 SEDIMENT CHEMISTRY AND WATER SAMPLES

The sediment samples from Lake Kirkkojärvi (Paper I) and from Lake Kivijärvi (Paper III) were analysed for selected elements (21 samples for Zn; Paper I, and 13 samples for P, Si, Fe, Mn, Zn, Ni, K, Mg, Na and S; Paper III) and geological characteristics (water content; Paper II and LOI; Paper III).

The sediment geochemical analyses for acid (HNO3) soluble (in accordance with EPA3051 and SFS-EN ISO 11885:2009 standard) concentrations were conducted by the Metropolilab environmental laboratory FINAS T058. Water content for Lake Kirkkojärvi samples was calculated by comparing sample weights before and after the freeze drying treatment. For Lake Kivijärvi sediment samples, LOI was measured by weight loss of dried samples (19 hours at 105 °C) after 3 hours of ignition at 550 °C (Heiri et al. 2001). In order to assess the current Lake Kirkkojärvi water chemistry, water samples were analysed for total phosphorus (TP), total nitrogen (TN), pH, soluble copper (Cu), nickel (Ni), and aluminium (Al) in the Metropolilab environmental laboratory (Paper II). In addition, previously retrieved sediment data (metals data; Parviainen et al. 2012 and diatom-inferred phosphorus data; Kihlman & Kauppila 2010) was used in the Lake Kirkkojärvi study (Paper I). Water monitoring data retrieved from an open database administrated by Finland’s Environmental administration (OIVA database 2018) was used in all studies.

2.2.4 DATING AND ARCHIVAL RESOURCES

In order to have chronological control of the sediment cores (Papers I and III), the sediment samples were radiometrically dated and the age models were calculated by the Liverpool University Environmental Radioactivity Laboratory, UK. In short, samples were freeze dried and sent to Liverpool University for 210Pb, 226Ra, 137Cs and 241Am (Paper III) or 210Pb, 226Ra and 137Cs
(Paper I) analysis by direct gamma assay. Chronologies were calculated by using the Constant Rate of Supply (CRS) model (Appleby 2001). In all studies, historical maps and archival data was used to assess the catchment land-cover history, water level changes and the mining activity in order to link the sedimentary data to the archival environmental history of the study site.

2.2.5 STATISTICAL METHODS

Principal components analysis (PCA) was used to visualize the general trends in cladoceran assemblages through time in the palaeo studies (Papers I and III). Non-metric multidimensional scaling (nMDS) analysis was conducted in the Lake Kirkkojärvi bay study (Paper II) where the focus was in the spatial impact of AMD and the differences between the pre- and post-mining cladoceran communities. Analysis of similarity (ANOSIM) was applied to test the significance of differences between pre- and post-mining communities in the Kirkkojärvi bay (Paper II) and in Lake Kivijärvi (Paper III). A Bray-Curtis dissimilarity matrix was used in the ANOSIM and nMDS analyses. The correlations between the distance from the tailings and water depth and the cladoceran assemblages was tested by using Pearson’s correlation. In the Lake Kirkkojärvi study (Paper I), the statistically significant zones in the cladoceran stratigraphy were identified by using the constrained sum of squares approach (Birks and Gordon 1985) and the broken stick model by Bennett (1996). Rarefied species richness and Shannon diversity were calculated in all studies by using count data, whereas square-root (Papers I and III) and/or log (x+1) (Papers II and III) transformations were applied to percentage data prior to multivariate analyses. All analyses were performed using PAST statistics 3.1 and 3.06 (Hammer et al. 2001), ZONE 1.2 (Lotter and Juggins 1991), and CANOCO 5.01.
2 RESULTS AND DISCUSSION

2.1 CHRONOLOGY

In Lake Kirkkojärvi (Paper I), the unsupported $^{210}\text{Pb}$ was abruptly lost from the record at the depth of 22 cm, whereas the distinct peak of $^{137}\text{Cs}$ (Chernobyl accident, 1986) was detected at the depth of 18-18.5 cm. The abrupt fall of $^{210}\text{Pb}$ is probably related to dilution by mined material (McDonald and Urban 2007), as the sediment section at the depth of 21.5-30 cm is characterized by dense material most likely originating from the mining activities. In Lake Kirkkojärvi bay (Paper II), the sediment surface samples were representing post-mining samples, whereas the pre-mining samples were obtained below the visible section of tailings material and thus are most likely older than the tailings release. The Kivijärvi sediment core (Paper III) exhibited the highest values of unsupported $^{210}\text{Pb}$ at the depth of 3 cm and a clear peak of $^{137}\text{Cs}$ and traces of $^{241}\text{Am}$ were detected at the depths of 4.5-4.75 cm pinpointing the Chernobyl accident in 1986. The lack of unsupported $^{210}\text{Pb}$ in the top samples is probably caused by dilution by the mined material (McDonald and Urban 2007).

2.2 HOW HAVE CLADOCERAN COMMUNITIES BEEN AFFECTED BY THE MINING ACTIVITIES AND POST-MINING AMD? (PAPER I)

In Lake Kirkkojärvi, a total of 32 cladoceran taxa were identified from 32 sediment samples (Paper I, Supplementary material). The cladoceran community consisted of species, which are common in European lakes (Bjerring et al. 2009). The most abundant species throughout the sediment record was *Eubosmina longispina* with an average relative abundance of 64 % (SD 11 %) followed by *Bosmina longirostris* 11 % (SD 11 %) and *Chydorus sphaericus* s.l. 5 % (SD 3 %) (Paper I; Fig. 3). Species richness and species diversity exhibited distinct changes throughout the sediment record and the values varied between 9.7 and 19.7 (average 14.8) for species richness and between 0.7 and 1.9 (average 1.4) for species diversity. The cladoceran stratigraphy was divided into three zones (Fig 5A), which are also clearly visible in the PCA biplot (Paper I; Fig 4). A distinct peak of sedimentary Zn
(680 mg/kg) was detected at the depth of 21.5 cm (record average 252 mg/kg) and the sediment layer between the depths of 19.0 cm – 31.0 cm was characterized by low water content (Fig 5A).

The cladoceran community structure and the sedimentary Zn concentration exhibit only minor variations in Zone III samples (44.5 cm – 32.5 cm, early 19th to early 20th century). Thus, Zone III most probably represents pre-mining conditions. The most distinct change in the cladoceran community in Zone III is the decreasing proportional abundance of *Eubosmina coregoni*, which is regarded as a pelagic species (Walseng et al. 2006) and prefers larger lakes. The decline of *E. coregoni* may be related to the water level lowering of Lake Kirkkojärvi during the 19th century, which resulted in the loss of the waterway and thus the migration route of cladocerans from the bigger Lake Kyrösjärvi to Lake Kirkkojärvi (Paper I; Fig 1).

The samples with the lowest species diversity and species richness values are found in samples of Zone II (30.5 cm – 23.5 cm, early 20th – early 1970s). The sediment is characterized by low water content, clastic inputs (titanium (Ti), potassium (K), magnesium (Mg), aluminium (Al), and vanadium (V)) and minor metals enrichment due to mine tailings dumping (Parviainen et al. 2012). In addition, some signs of changing environmental conditions corresponding with these sediment samples were also recorded by Kihlman and Kauppila (2010) as the diatom community exhibited temporary and minor, but clearly identifiable shifts. The low cladoceran diversity and species richness in Zone II indicates deteriorated environmental conditions, most probably due to fine grained tailings pollution, which impacted the lake at least until 1950-55 (Pöyry 2015). In general, the solids pollution has been acknowledged to have harmful impacts on cladocerans (EIFAC 1965; Garrido et al. 2003; Maia-Barbosa & Bozelli 2005; Bilotta & Brazier 2008; Kunz et al. 2013). Elevated concentration of particulate matter may hamper the cladoceran feeding efficiency (Arruda et al. 1983; Kirk 1992; Maia-Barbosa & Bozelli 2005) and turbidity-induced changes in the phytoplankton community may affect the food availability (Grobbeelaar 1985). The food quality is among the most important factors regulating the growth and reproduction of zooplankton (Groeger et al. 1991).

Zone I (22 cm – 0 cm, early 1970s to 2016) exhibits a clear change in the cladoceran community as the relative abundance of *B. longirostris* increases. The peak of Zn and the highest concentrations of other mining-related
contaminants (including sedimentary concentrations of Cu 3600 mg/kg, Ni 114 mg/kg, As 22 mg/kg; Parviainen et al. 2012) were found at the beginning of Zone I (1970s) highlighting the most intensive phase of AMD. Similarly to sedimentary concentrations, the water sampling results (OIVA database 2018) show higher metal concentrations in the 1970s when compared to the 1960s. This further suggests higher metal contamination during the years of the intensive AMD when compared to the active mining period, which was characterized by a direct dumping of waste. Interestingly, the samples at the beginning of Zone I (at the depth of 23 cm) exhibit clear recovery in terms of species diversity and species richness despite the highest concentrations of metals in the sediments. Moreover, the clearest change in the species assemblages is the appearance of *B. longirostris*, which is regarded as a species with low tolerance of copper (Koivisto et al. 1992; Koivisto & Ketola 1995; Bossuyt and Jansen 2005). This supports the observation by Kihlman and Kauppila (2010) that bioavailability of metals in the lake water was limited during the AMD. The reason for low bioavailability is most probably related to rapid dilution of mine water in the wetland area, which is located between the tailings and the lake. The dilution is visible in the declining metal concentrations and acidity in the water chemistry samples spanning from the tailings to Lake Kirkkojärvi (Parviainen 2009). The increasing pH accompanied by oxidizing conditions induces the precipitation of metals resulting in a decline of their solubility and toxicity (De Paiva Magalhães et al. 2015; Wang et al. 2016a). Further, organic ions (e.g. humic or fulvic acids) act as metal ligands, thereby decreasing the concentration of the soluble metal fraction in the water (e.g. Gaffney et al. 1996; Lalas et al. 2018) and reducing the metal toxicity (Paulauskis and Winner 1988).

Our results suggest that the tailings input caused a clear loss of cladoceran species richness and diversity. Low species richness contributes negatively to community resilience (Downing & Leibold 2010; Downing et al. 2014; Symons & Arnott 2014) and increases the vulnerability to community change (Folke et al. 2004), which in Lake Kirkkojärvi was observed as an increased abundance of *B. longirostris*, a species able to utilize both littoral and pelagic habitats (Adamczuk 2012). Thus it is capable of filling varying niches after the environmental disturbance. In addition, *B. longirostris* is regarded as a disturbance-tolerant species (Hart 2004) and exhibits rapid adaptation to changing environmental conditions (Adamczuk 2016). The gradual eutrophication detected in Lake Kirkkojärvi (Kihlman & Kauppila 2010) may also have contributed to the success of *B. longirostris* (e.g. Goulden 1964;
Korhola 1990; Nevalainen and Luoto 2013). However, because the phosphorus concentrations remained relatively stable since the mining period until the mid-1980s (Kihlman & Kauppila 2010), eutrophication alone cannot explain the appearance of *B. longirostris*. In general, the post-disturbance recovery of aquatic systems is a complex issue and the original species composition is not always re-established (O’Neill 1999). This seems to be the case in Lake Kirkkojärvi as well.

2.3 HOW DID THE CHRONIC BUT LOW-VOLUME AMD IMPACT THE CLADOCERAN COMMUNITIES IN A SEMI-ENCLOSED SYSTEM? (PAPER II)

The Lake Kirkkojärvi bay sediment stratigraphies were generally similar, with a distinct section of organic matter on the top of the core followed by black tailings and watery clay layers. The sediment below the tailings layer was brown lake gyttja (Paper II; Fig 2). In Lake Kirkkojärvi bay and the sediment samples from the reference sites, a total of 38 cladoceran taxa were identified. The species richness in Lake Kirkkojärvi bay samples was slightly higher in the pre-mining samples when compared to modern samples. The pre-mining community was not similar with the modern community (ANOSIM R=0.9081, p < 0.01) and the species composition in the Kirkkojärvi bay pre-mining community resembled the contemporary community of the Lake Viljakkalanselkä R2 “low agriculture” -reference site, whereas the contemporary species composition in Kirkkojärvi bay resembled the contemporary community of the Lake Kirkkojärvi R1 “high agriculture” -reference site (Fig 5B; Paper II; Table 3). There was no signal of AMD impact in post-mining communities. This was indicated by the clearly increased proportion of *B. longirostris*, which is sensitive towards copper pollution (Koivisto et al. 1992; Koivisto & Ketola 1995; Bossuyt and Jansen 2005) and, in Finland, is replaced by *E. longispina* in acidified systems (Uimonen-Simola & Tolonen 1987). It should be noted though that community change is not systematic when acidity preferences of cladoceran species are considered. For example, the relative abundances of *Monospilus dispar*, *Disaparalona rostrata*, *Rhyncotalona falcata* and *Alonella excisa* are lower in the modern samples compared to the pre-mining samples, but according to Krause-Dellin & Steinberg (1986), *M. dispar* and *D. rostrata* are alkaliphilous species, whereas *R. falcata* and *A. excisa* are acidophilous.
In addition, there was no clear trend regarding the cladoceran assemblages and the distance to the AMD input. *Alonella nana* was the only species which exhibited higher proportional abundances at the sites near the AMD input. However, other taxa, which are well known to thrive in acidic and shallow systems (e.g. acidic peatland lakes; Drinan et al. 2013) did not show a similar preference in Lake Kirkkojärvi bay. Moreover, *A. nana* was highly abundant in the Lake Kirkkojärvi R1 reference site, which is unaffected by the AMD. Thus, the high abundance of *A. nana* could be related to other environmental factors than AMD, such as e.g. high coverage of aquatic vegetation (Bjerring et al. 2009; Adamczuk 2014). Also the metal concentrations in the water samples retrieved from Lake Kirkkojärvi bay were low and did not exhibit an AMD impact (Paper II, Table 2).

The pH in the tailings ditch, located at the waste area, was 3.5 and at the head of the bay pH was 6.8 (Paper II; Table 2). Thus, the lack of AMD impact in the shallow bay is probably related to the relatively low volume of the effluent, which is efficiently diluted when entering larger water volumes as suggested earlier by Parviainen (2009). Even though the metal speciation (and subsequently their toxicity) in fresh waters is a complex phenomenon (De Paiva Magalhães et al. 2015), some general considerations can be made. pH is noted as being the most important variable in controlling the behaviour of metals in aquatic systems (USEPA 2007). Generally, increasing pH results in precipitation of metals, which, in many cases, reduces their bioavailability (John & Leventhal 1995; De Paiva Magalhães et al. 2015). In addition, organic ions (e.g. fulvic and humic acids) act as metal ligands and together they form complexes, which are less bioavailable for cladocerans (e.g. Copper; Kramer et al. 2004). Humic substances originate from decomposition of plant and animal tissues (e.g. Gaffney et al. 1996) and are thus probably present in the vegetated wetland of Lake Kirkkojärvi. In fact, wetlands have high potential for the treatment of acidic mine drainage (Ditsch & Karathanasis 1994) and wetland systems have been constructed to remediate acid mine drainage (Hallberg & Johnson 2005). Further, in Lake Kirkkojärvi bay, the shallow wetland (< 1 m, 1.6 ha) vegetation is dominated by *Equisetum fluviatile*, which has been shown to accumulate metals (Bateman 1999). Yet another explanation for the lack of AMD impact in the cladoceran communities is related to the timing of the AMD input. Most probably, the highest volume of drainage occurs during the spring snowmelt, when the cladoceran community is still dormant (e.g. Koksvik 1995). Even if the overwintering population and the resting stages are affected by spring AMD, the population is likely re-
established quickly by the cladocerans migrating from the Lake Kirkkojärvi main basin (Louette & De Meester 2005).

The modern cladoceran community structure is similar in Lake Kirkkojärvi bay and at the Lake Kirkkojärvi reference site. Thus, even though the reference sites were not sampled for pre-mining conditions, it is likely that the cladoceran community has changed all around Lake Kirkkojärvi and not only in the bay adjacent to tailings (Fig 5B, Paper II; Table 3). This is further confirmed by results presented in Paper I (Paper I; Fig 3), where the proportional abundance of *B. longirostris* is clearly higher in modern samples, when compared to pre-mining samples. Most probably, Lake Kirkkojärvi resembled Lake Viljakkalanselkä during the pre-mining period. Based on cladoceran assemblages (*B. longirostris* is regarded as an indicator of eutrophication (e.g. Goulden 1964; Korhola 1990; Nevalainen & Luoto 2013)) and nutrient enrichment trends shown by Kihlman and Kauppila (2010), the most important difference between the pre- and post-mining conditions in Lake Kirkkojärvi is related to lake trophy. However, even though the AMD does not seem to affect modern cladoceran species assemblages in Lake Kirkkojärvi bay, the results presented in Paper I suggest that the initial reason behind the community change was indeed related to mining.

2.4 HOW DID THE SALINE MINE WATER WITH ELEVATED METAL CONTENT IMPACT THE CLADOCERAN COMMUNITIES IN A BOREAL LAKE? (PAPER III)

The mine waste water has clearly changed the water chemistry of Lake Kivijärvi (Paper III; Fig 2). For example, the surface water (0-4 m) sulphate concentrations have increased from a pre-mining average of 1.5 mg/L (SD 0.28 mg/L, N=2) to an average of 617 mg/L (SD 518 mg/L, N=61) between 2008 and 2015. Similarly, Ni and Zn concentrations have increased from 0.5 μg/L (N=1) and 5 μg/L (N=1) to 52.6 μg/L (SD 93.3 μg/L, N=44) and 35.9 μg/L (SD 39.0 μg/L, N=44), respectively (OIVA database 2018). In addition, the mining impact can clearly be seen in the sediment chemistry (Fig 5C, Paper III; Fig 3), where multiple mining-related elements show elevated concentrations at the samples near the sediment surface: Ni and Zn peak at the depth of 1 cm – 0.5 cm, which most probably pinpoints the Talvivaara dam accident in the late 2012.
The cladoceran and diatom communities clearly changed due to the impact of the mining pollution. The most important changes at the species level are the shift from *Daphnia cucullata* dominance to *Bosmina longirostris* dominance in the cladoceran community and the shift from the dominance of *Aulacoseira subarctica* to the dominance of *Diatoma tenuis* in the diatom community. The shifts in species assemblages are demonstrated also in the species diversity and species richness indices (Fig. 5C), species stratigraphies (Paper III; Fig 5 and Fig 6), and PCA biplots (Paper III; Fig 4). These shifts can additionally be seen in the results of the analysis of similarity between the pre- and post-mining sediment samples, which show significant differences for both indicator groups (ANOSIM $R = 0.814 \ p < 0.01$ for cladocera and $R = 0.928 \ p = 0.017$ for diatoms). Moreover, species richness and species diversity have decreased in both groups due to the mining impact (Fig 5C).

The increased abundance of *B. longirostris*, which in Europe is regarded as a sensitive species to metals pollution, (e.g. Cu; Koivisto et al. 1992; Koivisto & Ketola 1995; Bossuyt & Jansen 2005) suggests only a minor impact of metals. Generally, the bioavailability of metals decreases due to elevated hardness (Paulauskis and Winner 1988; Heijerick et al., 2002; Deleebeeck et al., 2007) and the increased calcium (Ca) concentrations in the lake water (Paper III; supplementary material) may thus explain the success of the metal-sensitive taxa. The sulphate pollution is more likely to explain the community changes, as both indicator groups exhibit changes, which can be connected to elevated salinity. The cladoceran *B. longirostris*, which has dominated the cladoceran community during the recent years has been noted to tolerate a wider range of salinity than many other cladoceran species (Aladin 1991; Paturej & Gutkowska 2015), and it has been recorded in environments with higher salinity when compared to e.g. *Daphnia* (Jeppesen et al. 1994; Boronat et al. 2001; Amsinck et al. 2003). In fact, *D. cucullata*, a species, which nearly vanished from the lake system, is regarded sensitive to elevated salinity within its genus *Daphnia* (Gonvalaves et al. 2007). The strong dominance of the diatom species *Diatoma tenuis*, which is known to thrive also in brackish waters (Snoeijs 1993), suggests that diatom communities, too, are affected by the clearly elevated salinity.

In addition to the osmotic stress induced by the elevated salinity, meromixis and subsequent deep water anoxia has changed the quality of the deep water...
The sediment contamination adds to the harmful impacts of pollution (Pereira et al. 2000) due to the direct contact with benthic species (Dekker et al. 2005) and due to the negative effects on cladoceran resting eggs deposited in the sediment (Rogalski 2015). In the diatom community, the proportion of benthic species has decreased, which suggests deteriorated conditions in the benthic habitat. The Lake Kivijärvi case is a complex multi-stressor problem, where the biological communities are simultaneously affected by meromixis, osmotic stress and metal pollution. However, based on the ecological preferences of the indicator groups used, increased salinity can be interpreted as the critical component in the mine water, which has induced the changes in the aquatic ecosystems of Lake Kivijärvi.

Figure 5. General results. A: Lake Kirkkojärvi core (Paper I). H' denotes cladoceran diversity, R denotes cladoceran richness. W (%) is water content and Zn (mg/kg) is sedimentary Zn concentration. Horizontal lines represent the zones I-III. B: Lake Kirkkojärvi bay nMDS plot (Paper II). C: Lake Kivijärvi core (Paper III). Gray section marks the mining era (2008-2015). Cla R and Cla H are cladoceran richness and diversity, respectively, whereas Diat R and Diat H are diatom richness and diversity, respectively. Na (mg/kg) and S (mg/kg) are sedimentary concentrations for Na and S, respectively. Ni (mg/kg) and Zn (mg/kg) are sedimentary concentrations for Ni and Zn, respectively.
2.5 CLADOCERANS AS SENTINELS OF MINING POLLUTION

The results of this work strongly support previous findings that cladocerans are sensitive to aquatic pollution and are highly useful as an early warning palaeobioindicator group in pollution studies. The applicability of cladocerans as pollution sentinels is based on two characteristics: Firstly, cladocerans are regarded as one of the most sensitive groups towards pollution (e.g. metals; Brix et al. 2001; Von Der Ohe & Liess 2004, chloride; Corsi et al. 2010, sulphate; Wang et al. 2016b). Secondly, as Cladocera have a central location in the food-web, even small changes in their species composition may resonate through the aquatic ecosystem (Carpenter et al. 1985) and thus can affect tolerant species that were not originally impacted by the initial pollution per se. In this work, the important planktonic species (*Daphnia cucullata*, *Eubosmina longispina* and *Bosmina longirostris*) exhibited distinct shifts due to pollution: larger-sized *D. cucullata* and *E. longispina* were replaced by the small *B. longirostris*. The importance of this observation is related to the fact that a diverse size structure of the zooplankton community enhances the strength of grazing (Sommer and Sommer 2006; Ye et al. 2013). Moreover, because large filter feeders such as *Daphnia* have a central role in enhancing the water clarity (Scheffer 1999), and different cladoceran species exhibit varying abilities to graze on cyanobacteria (Tonno et al. 2016), cladocerans have a direct connection to water quality issues. Moreover, because vendace (Viljanen 1983; Helminen et al. 1990; Eckmann et al. 2002) feeds extensively on large-sized cladocerans, human-induced changes in cladoceran size structure may affect the food availability for commercially important fisheries.
3 CONCLUSIONS AND FUTURE PROSPECTS

The main conclusions based on the results of this thesis are:

1. The tailings dumping affects the cladoceran community by reducing their diversity and species richness. The post-mining AMD signal could not be seen in the cladoceran community and may have been masked by eutrophication.

2. The impact of chronic AMD was not visible in the cladoceran community even in the vicinity of the AMD inflow. The lack of the impact of pollution is most probably related to the low volume of AMD, which is rapidly diluted.

3. Biomining effluent has a potential to cause distinct changes in cladoceran and diatom communities. The main stressor affecting the community change in Lake Kivijärvi is the salinity of the mine water.

The most important finding of this work is the fact that the strongest negative impacts of mine water originating from metal mines are not necessarily related to metals or acidity but to rapid changes in environmental conditions due to input of mineral tailings (e.g. Lake Kirkkojärvi) and to increased salinity (Na₂SO₄; e.g. Lake Kivijärvi). These substances are usually not considered toxic and thus the problems related to these contaminants may be underrepresented, when treatment solutions, mitigation obligations and waste water quality thresholds are considered. In Finland, for example, sulphate is not included in the list of government decree on substances, which are dangerous or harmful to the aquatic environment (Decree 1022/2006). The results of this work highlight the importance of “non-toxic pollutants”, such as saline drainage, when mining impacts are assessed.

The sensitivity of littoral species as indicators of water pollution is not completely resolved and thus it is difficult to name early warning species amongst littoral cladocerans. Palaeolimnological methods applied to appropriate sites with distinct pollution gradients could be used to fill this gap. Laboratory assays and in-situ testing could be used to complement the palaeolimnological studies. Moreover, because cladocerans are regarded as
lake biota, the group has usually not been used in river research even though mine waters are usually channelled into lentic systems. This is clearly an interesting research area, which could provide new insights into mining pollution of rivers with potential applications for management and restoration of these environments.
ACKNOWLEDGEMENTS

You know, the working title for this thesis was “Dark Satanic Mills and The Earths Open Wounds”, copied from William Blake’s poem (1808) and from an article by Fields (2003). It never made it to the cover, but now it is acknowledged on the first line of this section. Mission accomplished.

This has been a joy. I have been extremely lucky to circumvent big obstacles and my work did proceed as planned. Large chunk of this luck is not actually luck at all but a result of the quality of the guidance and help I was receiving through this PhD project. The help I got from my supervisor Dr. Jan Weckström was clearly the most important thing in this whole process. For this, I am truly thankful. During these 4 years our discussions gradually shifted from research towards other issues, such as football. I suppose and hope, that this reflects my learning: there was time to talk about other issues than research! Professor Atte Korhola, my second supervisor also deserves my most honest gratitude for seemingly never ending drive and support! Thank you dear thesis committee, O-P, Ulrika and Maija for your advice and encouragement!! Pre-examiners Jennifer Korosi and Tommi Kauppila, thank you so much for your time and suggestions on how to improve this thesis! Special thanks for Kaarina for comments regarding the thesis! I would also like to thank everybody in our research group for you kindness and help along the way. Minna, Tarmo, Kaarina, Maija, Sari, Meri, Marttiina, Sanna K., Sanna P., and Tiia. Hui, I have never really expressed my thanks for the peer support and advice! Thank you so much!! Let’s keep in touch. Marco and Paul, you were nearing your defence dates when I started my PhD project. It was nice to meet you here at Viikki. Thanks for Annika Parviainen for your help and co-operation! Thanks to Schulte-Tigges family for help, accommodation and excellent company during the Lake Kirkkojärvi fieldwork! And surely; the dearest Linda, Vilma, Otto and Veikko. My brothers, and their families, my parents, and other relatives and friends. Thank you for support and interest in my work! If I missed somebody, please forgive me. I’ve been in a hurry lately putting this thing together.

But this 4 years has not been entirely about research. There is life outside work, you know, and there are hobbies too. During these 4 years I have learned
to freedive a bit and to do martial arts a bit. It has been great, I’ve made many new friends. The soundtrack of this phase of my life sounds like Killer Mike, Talco and Hollie Cook.

This thesis was funded by Tellervo and Juuso Walden Foundation & The Soil Protection and Environmental Technology Association (MUTKU) & The Kainuu Centre for Economic Development, Transport and the Environment & K.H. Renlund Foundation & Societas Pro Fauna et Flora Fennica.
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