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NiSO₄ spill inflicts varying mortality between four freshwater mussel species (including protected *Unio crassus* Philipsson, 1788) in a western Finnish river

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Abstract

Freshwater mussels are one of the most threatened taxonomic groups in the world, and many species are on the brink of local or global extinction. Human activities have altered mussel living conditions in a plethora of ways. One of the most destructive human-induced impacts on running waters is the catastrophic spill of harmful substances, which results in massive die-offs. Even though Finland is regarded as the world's top country in terms of environmental regulation quality, riverine systems are not safe. In 2014, River Kokemäenjoki in western Finland experienced the worst NiSO₄ spill in the country's history, visibly affecting the mussel community – including protected *Unio crassus* – along the river. Because freshwater mussel toxicology is grossly understudied (particularly in Europe), any pollution –linked die-offs offer valuable opportunities to study the issue in natural environment. Here, we report the mussel investigations from 2014 and a follow-up study conducted in 2017 in order to assess the variation in species sensitivity on nickel pollution. In total, 104 sites were sampled, and over 20 000 mussels were identified and counted. Our results

indicate that the most impacted species (i.e. that which experienced the highest spill-induced mortality) was *Anodonta anatina* (62%), followed by *Unio pictorum* (32%), *U. crassus* (24%) and *Unio tumidus* (9%). The underlying reason for the sensitivity of *A. anatina* is not resolved, hence more research is urgently needed. The low mortality among most of the species in 2017 highlights the temporal nature of the pollution impact and the recovery potential of the mussel community. However, the case is more complex with *U. crassus* population, which may be experiencing delayed impacts of the spill. Because nickel is one of the most commonly produced industrial metals in the world (hence the pollution incident risk is high) and River Kokemäenjoki hosts mussel community typical for European rivers, our results may benefit many researchers and stakeholders dealing with riverine environments.

Main finding: The freshwater mussels exhibit differences in NiSO₄ pollution tolerance. *Anodonta anatina* is the most sensitive species, followed by *Unio pictorum*, *Unio crassus* and *Unio tumidus*.

Keywords: *Unio crassus*, *Anodonta anatina*, metals, pollution, freshwater mussels, Finland

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1. Introduction

The majority of the world's riverine ecosystems are threatened by human activities (Vörösmarty et al. 2010), thus the state of running waters is a pressing and global concern. Riverine systems are affected by agricultural and urban land use, industrial activities, watercourse alterations and direct species introduction or removal (Malmqvist and Rundle 2002). In addition to the chronic environmental impact inflicted by many of the aforementioned activities, single chemical spills or other isolated incidents may also pose high threats to riverine ecosystems. Even though the impact of a single spill on water quality is usually temporally limited due to water flow, the biota may still be greatly impacted over longer timeframes (Crossman et al. 1973; Soldan et al. 2001; Giger 2009).

Some of the most important invertebrates in rivers are riverine mussels, which contribute to water quality, nutrient cycling and abundance of other benthic organisms (Nobles and Zhang 2011). Unfortunately, many mussel species are endangered due to habitat destruction (e.g. dredging, siltation), declining water quality (e.g. contaminants, eutrophication) and loss of host fish communities (e.g. due to dams) (Williams et al. 1993). According to a recent review, nearly 40% of freshwater bivalve species are near threatened, threatened or extinct, putting them on the top of the list of most threatened taxonomic groups in the world. The situation is

most drastic within the order Unionidea: 25 species (2.8%) are confirmed or presumed to be extinct (Lopes-Lima et al. 2018). In order to protect aquatic ecosystems, the European Union is committed, via the Water Framework Directive, to the ambitious goal of ensuring the good quality of surface waters by 2027. Furthermore, two freshwater bivalve species are currently protected within the European Union (*Unio crassus* Philipsson, 1788 and *Margaritifera margaritifera* Linnaeus, 1758; EEC 1992). However, knowledge of the impacts of environmental pollution on adult Unionoid species in European rivers is still scarce, as most of the laboratory studies are conducted on North American species (Havlik and Marking 1987; Beggel et al. 2017). Moreover, laboratory bioassays are conducted in controlled environments, hence the results obtained in these laboratory experiments do not necessarily reflect what would occur in nature. Therefore, any large-scale mussel die-off – especially where the cause and timing are identified – has potential to provide highly important data regarding the sensitivity differences between species, and overall community recovery dynamics. These environmental incidents can provide researchers with a valuable opportunity to study ecotoxicological effects in a natural setting. Such information is crucial in order to protect threatened species, and to offer insight into the sustainable management of rivers.

Nickel is widely utilized industrial metal with great importance on e.g. stainless steel manufacturing (Reck et al. 2008). However, nickel release originating from many anthropogenic sources, e.g. mines (Leppänen et al. 2017) and smelters (Woodfine and Havas 1995) is known to inflict serious damage to aquatic environment. Finland, which ranked highest (out of 75 countries) in the national environmental regulation quality assessment (Esty and Proter 2002), experienced the country’s largest industrial nickel (Ni) spill accident in July 2014. The spill entered River Kokemäenjoki, which is inhabited by five freshwater mussel species, including the thick-shelled river mussel, *Unio crassus*. This protected species (EEC 1992) is listed as endangered due to the 50% decline across its range within the past 50 years (Lopes-Lima et al. 2014). The other species inhabiting the river include *Unio tumidus* Philipsson, 1788, *Unio pictorum* Linnaeus, 1758, *Anodonta anatina* Linnaeus, 1758 and *Pseudoanodonta complanata* Rossmässler, 1835.

The main hypothesis in our study is that mussel species exhibit variation in Ni sensitivity resulting clear differences in spill-induced mortality. Since there is a considerable research gap in pollutant sensitivity among European freshwater mussels, the results of this study are very important for authorities dealing with river management and pollution assessment of freshwaters.

2. Materials and methods

2.1 Study site

River Kokemäenjoki is a 120 km long river in western Finland, with an average discharge of 223 m³ s⁻¹. The river is the fifth largest in Finland; the size of the catchment area is 27 046 km² (KVVY 2015), which consists

106 mostly of forests (57%), agricultural fields (14%) and water (11%). There is one major city (city of Pori, with
107 ~85 000 inhabitants) and several other smaller towns located by the river (Fig 1). Based on water monitoring
108 data (OIVA Database 2019), the river water (sampled at the city of Pori) is soft (Ca average 8 mg L⁻¹ N = 7;
109 Mg 2.4 mg L⁻¹ N = 1), nearly neutral (median pH 7.2 N = 385) and classified as eutrophic (total P average 45.7
110 µg L⁻¹ N = 375; total N 1288 µg L⁻¹ N = 378). The average electric conductivity is 11.6 mS m⁻¹ (N = 282). The
111 water quality has improved considerably during the past decades due to improved industrial waste management
112 practices (KVVY 2015). However, despite these improvements in waste water management, the river is still
113 impacted by point and nonpoint sources of water pollution. In addition, due to the high industrial activity by
114 the river, there is still considerable risk of environmental accidents. One of such incidents was the Norilsk
115 Nickel nickel sulphate (NiSO₄) spill in July 2014, which, according to environmental officials, was the largest
116 ever Ni spill in the history of Finland. In total, 66 tonnes of Ni and 94 tonnes of SO₄ were released into the
117 Harjavalta hydropower plant reservoir, located at River Kokemäenjoki. Elevated Ni concentrations were
118 measured for ~20 days after the spill (KVVY 2016; Fig 2). The highest Ni concentration measured after the
119 leak was 8 700 µg L⁻¹ (KVVY 2015), and even though the Ni concentrations declined rapidly, high numbers
120 of dead mussels (soft tissue) were reported along the river.

121

122 2.2 Sampling

123

124 We used mussel mortality data from 2014 and 2017 to assess the impact of the spill on the mussel community
125 along the river and available water monitoring data to assess the spatial and temporal changes in water quality
126 (i.e. the general dynamics of Ni pulse) in the river, downstream of the spill site. The mussel species assemblage
127 and recent (spill-induced) mortality was analyzed from 48 downstream sites and 10 upstream reference sites
128 after the spill (7 July–12 September 2014), and again in 2017 from 39 downstream sites and 7 upstream
129 references sites (between 11 June and 18 August 2017). Transects were placed at approximately 1 km intervals
130 between the spill site and the sea. The site locations were checked on site and moved if sampling was deemed
131 not possible (e.g. dangerously high current velocity). The first transect start was randomly selected (but still
132 placed at an adequate location due to the hydropower dam) within the 1 km distance downstream of the spill
133 site (systematic sampling of mussels; Strayer and Smith 2003). In addition, a total of seven (five in 2014 and
134 two in 2017) qualitative mussel samples were collected where the transect area was not defined.

135

136 Due to the depth of River Kokemäenjoki, we used a scuba transect method to collect mussels. The method is
137 regarded as semi-quantitative because visual and tactile searches may miss small and/or buried mussels (Miller
138 and Payne 1988). No excavation samples were conducted, thus the detection probability is unknown. Briefly,
139 a diver proceeded along the pre-installed rope and collected all mussels and shells into a mesh bag. Mussels
140 were identified, classified and counted at the surface and subsequently returned to the original area. The
141 targeted minimum sample size/transect was set to 200 (see e.g. Leppänen 2018). Transect cover area data
142 (available from 53 sites in 2014 and from 44 sites in 2017) was used to calculate mussel density values. To

143 assess mussel mortality, we classified the mussels and shells using a modified post-mortem characteristics-
144 based scale developed by the Michigan Natural Features Inventory (see e.g. Badra 2011), which classifies the
145 mussels as either alive, recently dead, or worn based on the following characteristics:

146

147 Alive: shell tightly shut when exposed to air.

148

149 Recently dead: soft tissue present or absent; hinge ligament intact; inside shell not chemically altered; most
150 of the periostracum intact; shell has its original strength.

151

152 Worn: inner surface covered by periphyton and/or chemically altered; and/or hinge ligament worn; hinge or
153 shell strength distinctly altered.

154

155 To assess the spill-induced mortality, we calculated theoretical pre-spill (alive mussels + recently dead
156 mussels) community numbers. To analyze the post-mortem transport of mussel shells (i.e. whether the dead
157 mussels deposited at their site of origin, or transported downstream), we compared the theoretical pre-spill
158 community numbers with the worn shell numbers.

159

160 **2.3 Statistical methods**

161

162 We used Spearman's rank correlation (on non-normal count data) to test whether the dead mussels are
163 deposited at their site of origin (i.e. with significance of $P < 0.01$). Mortality hotspots were analyzed using
164 Getis-Ord G_i^* analysis (Getis and Ord 1992), using mortality percentages. Briefly, the analysis works by
165 assessing each site value within the context of the neighboring site values. High values are significant only if
166 they are surrounded by high-value neighbor sites. The threshold distance (Euclidean) was set as the average
167 distance at which each sampling site had 8 neighbors (Mitchell 2005). The Getis-Ord G_i^* analysis was
168 conducted in ArcMap 10.3.1 (ESRI 2015), and the correlation analysis was conducted with Past 3 statistical
169 software (Hammer et al. 2001).

170

171 **2.4 Population size calculation**

172

173 The number of individuals was calculated by interpolating the mussel densities in the systematically placed
174 transects (transects 820–36078; only the NE channel in the Seaward sites was included) to the river segments
175 between them. The average mussel density in the transects at the ends of a segment was multiplied by the area
176 of the segment. The total numbers of mussel species in each segment were added together for the total
177 population size in the impacted area.

178

179 **3. Results**

3.1 Water pollution

The highest measured total Ni concentration in River Kokemäenjoki (8700 µg L⁻¹ Ni) was recorded near the spill site on 7 July 2014 in deep (19 m) water (KVVY 2015); the deep water concentrations remained above 250 µg L⁻¹ for seven days near the spill site. Background concentrations were reached 20 days after the spill (KVVY 2015). Based on water monitoring data obtained from ELY (2019), the highest surface water concentrations were measured on 7 July near the spill site (2700 µg L⁻¹), and on 8 July near the City of Pori (1700 µg L⁻¹ Ni). Surface (1 m) water concentrations remained elevated (values above the National Ni quality threshold index 20 µg L⁻¹; Vuori et al. 2009) for ten days after the spill (Fig 2). The average total Ni concentration (surface water) near the spill site before the accident was 1.9 µg L⁻¹ (2003–2013, N = 34) and 4.1 µg L⁻¹ (2012–2013, N = 11) at the City of Pori. The average total Ni concentration (surface water) near the spill site in 2017 was 1.7 µg L⁻¹, N=6 and 3.8 µg L⁻¹, N=6 at the City of Pori (OIVA Database 2019).

3.2 Mussel results 2014

In 2014, due to variation in river width, the transect lengths varied from 40 m to 185 m and transect width varied from 0.1 m to 1.5 m. The maximum water depth varied from 2 m to 13 m. The total number of identified mussels (alive mussels and empty shells) from all 58 sites was 11,762. The number of mussels per site varied from 33 (site -17000) to 511 (site -37700), and the average number of mussels per site was 202. Mussel density was highly variable between the 53 sites where the transect width was assessed (average 6.4 mussels per m², SD 6.3, range 0–27). The most abundant species (including alive, recently dead, and worn) was *U. pictorum* (3,581; 30%) followed by *U. crassus* (3,199; 27%), *U. tumidus* (3,178; 27%) and *A. anatina* (1,803; 15%). In addition, one specimen of *Pseudoanodonta complanata* was found, but was omitted from further analysis. The clearest difference regarding spatial variation among species was the high abundance of *A. anatina* in the reference sites, whereas in impacted sites, *Unio* species were more abundant. Among the impacted sites, all species showed roughly similar trends: lower densities at Small town sites, and higher densities and variation at City of Pori sites and Seaward sites (Fig 3). Background mortality (i.e. percentage of worn shells) exhibited high variation, and the only common character was the increased percentage of worn shells in the Seaward sites downstream from the City of Pori and in the reservoir located upstream from the spill site (Online Resource 1). Alive and recently dead mussels (theoretical pre-spill community) were positively correlated with worn shells (Spearman's r_s for *A. anatina* = 0.6, $P < 0.01$; Spearman's r_s for *U. crassus* = 0.6, $P < 0.01$; Spearman's r_s for *U. pictorum* = 0.7, $P < 0.01$; Spearman's r_s for *U. tumidus* = 0.7, $P < 0.01$). The recent average mortality (recently dead / alive + recently dead * 100) in sites downstream from the spill site (48 impacted sites) was highest for *A. anatina* (62%; SD 24%), followed by *U. pictorum* (32%; SD 14%), *U. crassus* (24%; SD 22%) and *U. tumidus* (9%; SD 8%) (Fig 4). Pre-spill (alive + recently dead) and post-spill (alive) densities for each species are presented in Table 1 and Fig 3. *A. anatina* exhibited the highest mortality

in 39 sites, followed by *U. pictorum*, which exhibited the highest mortality in six sites, and *U. crassus* in three sites. The recent mortality at sites located upstream from the spill site (reference sites) was low (average 0–2.6%). Based on population size calculations, the size of the pre-spill *U. crassus* community was nearly 6.5 million mussels, and the number of spill induced deaths was over one million.

Among the impacted sites, there was considerable variation in recent mortality. *A. anatina* exhibited slightly decreasing mortality from the spill site towards the sea, whereas *U. crassus* and *U. tumidus* showed the opposite trend. Recent mortality for *U. pictorum* did not show any distinct directional changes among the impacted sites. Mortality hotspot analysis detected significant ($P < 0.01$) mortality hotspots for *A. anatina* and *U. crassus* (Fig 3).

3.3 Mussel results 2017 and the post-spill recovery

The 48 follow-up transect samples in 2017 resulted in 11,401 mussels (30 worn and two alive *Pseudoanodonta complanata* were omitted from analysis). Among the 2017 samples, recently dead *A. anatina* were not detected, and the recent mortality was very low for *U. pictorum* (maximum 1.5%). For *U. tumidus*, there was one site where the recent mortality was clearly elevated (site 29803; 57% mortality) (Fig 5). *U. crassus* exhibited relatively low recent mortality in multiple sites located in the middle section of the river (maximum mortality at site 9335; 17% mortality) (Fig 5). Based on mean mussel densities (Table 1) *U. tumidus* exhibits most complete recovery in 2017 samples, whereas *A. anatina* and *U. pictorum* occur in nearly identical densities in post-spill 2014 and in 2017 samples. *U. crassus* shows continuous decline in mean density. In reference sites, maximum and average densities for *A. anatina* and *U. tumidus* are considerably higher when compared to 2014 results, whereas for other species, the densities are nearly similar. The abundance of worn mussels in 2017 resembled the abundance of recently dead and worn mussels in 2014 (Online Resource 1 and 2). According to our calculations, the current population size of *U. crassus* is nearly 5 million mussels.

4. Discussion

Our study clearly shows that NiSO_4 had varying impact on different mussel species. Because the mussel species identified in River Kokemäenjoki are typical riverine species in Europe (Lopes-Lima et al. 2017), and the density ranges closely agree with commonly reported values for these species (e.g. Weber 2005; Zajac et al. 2016), our results are highly relevant in European mussel ecosystems. Some of the reference sites exhibit low flow conditions (i.e. some sites resemble lake rather than a river) and the high density of *A. anatina* at some of the reference sites is likely attributed to slow flow conditions. The differences in current velocity between reference sites and impacted sites is most probably related to hydropower plant dam located near the spill-site and historical channel manipulation downstream the spill site. The Small towns-sites (starting below the spill site) seem to be less suitable for mussels, likely because of the high flow rates (Layzer and Madison 1994;

Strayer 1999) and higher flow variation due to the hydropower plant (high water release during working hours from peak in electricity demand). At the City of Pori, the flow velocity slows down (Lotsari et al. 2013), which may explain the higher mussel densities at multiple City of Pori and Seaward sites. Since mussels occur in mixed species mussel beds, it can be assumed that all species were equally exposed to the NiSO₄ spill along the river.

For example, *U. crassus* were considered to be mostly impacted by agricultural diffuse effects (Lopes-Lima et al., 2014), but recent studies have shown that they may be more tolerant to such factors than previously assumed (Stoeckl and Geist 2016; Inoue et al. 2017). This stresses the importance of understanding the effects of industrial pollution spills investigated in this study of the Kokemäenjoki. Our results resemble those of other high-magnitude riverine pollution incidents of instant deleterious effects on mussel communities (e.g. Crossman et al. 1973; Anderson et al. 1991). In River Kokemäenjoki, all species were impacted along the ~35 km stretch of the river. Interestingly, Getis-Ord Gi* analysis detected mortality hotspots (also visible in curves in Fig 3) for *A. anatina* in sites near the spill, and for *U. crassus* in Seaward areas. This finding suggests differences in the magnitude of NiSO₄ impact between species and sites. The mussel tissue analysis (Väisänen 2018; Online Resource 3) conducted in 2014, 2015 and 2017 showed high variation between species, tissues and sampling sites. The clearest spill –related observation was the concentration peak in the 2014 samples retrieved immediately after the spill (*Unio* spp. Ni 4.8 mg kg⁻¹ 2.1 SD mg kg⁻¹ wet weight, N=7, gills and muscle) and the rapid decline one and half months later (*Unio crassus* Ni 0.39 mg kg⁻¹ SD 0.27 mg kg⁻¹ wet weight N=12 and *A. anatina* Ni 0.36 mg kg⁻¹ SD 0.29 mg kg⁻¹ wet weight N=12, gills and muscle). Moreover, the analysis shows that mussel gills exhibit higher Ni concentrations when compared to muscles. There is an overall higher Ni concentration downstream from the spill site also in 2015 and 2017, but this is difficult to relate to the spill because the Ni concentration in river water was 2 times higher at the City of Pori when compared to spill site before the accident. Typically, the heavy metals concentrations vary among species, tissues and sampling sites (*A. anatina*, *U. pictorum*; Gundacker 2000; *A. anatina*, *U. tumidus*; Rzymiski et al. 2014). As such, the metals accumulation is not considered an ideal method for assessing the toxic impact (Zuykov et al. 2013). Typically, heavy metals uptake is first very rapid and then levels off. Similarly, once the environment is uncontaminated, the elimination of heavy metals is very rapid at first, followed by slower elimination (Thorsen et al. 2006), which explains the reported metals concentration peaks immediately after the spill and lower metals concentrations a few months later.

The mussel species did not exhibit any clear preferences in terms of habitats (patchy distribution and multi-species mussel beds is commonly reported; Watters 2006; Allen et al. 2013; Lopes-Lima et al. 2015), which could have been used to assess the reasons for differences in mortality. In addition, the filtration rates for *A. anatina*, *U. crassus*, *U. tumidus* and *U. pictorum* are nearly identical (Kryger and Riisgård 1988) and *A. anatina*, *U. tumidus* and *U. pictorum* exhibit similar activity levels and grain size preferences in pedal feeding (benthic material is taken up by the mussel foot and transported into the digestive system) (Brendelberger and

Klaue 2009). Subsequently, habitat preferences or variation in feeding/filtration activity do not seem to explain the mortality differences.

According to a recent review, the most important toxicity mechanisms of Ni on aquatic organisms are disruption of homeostasis of Ca^{2+} , Mg^{2+} and/or $\text{Fe}^{2+/3+}$, oxidative damage and an allergic –type response at respiratory tissues (Brix et al. 2016). In case of River Kokemäenjoki, multiple of aforementioned mechanisms may have simultaneously contributed to the observed negative impacts. There is a lack of toxicological thresholds for European *Unio* species regarding Ni (Naimo 1995; Farris and Van Hassel 2006), making it difficult to assess the tolerance variation between species. However, measured Ni concentrations exceed the acute toxicological thresholds for juvenile North American *Unio* species ($173\text{--}676\ \mu\text{g L}^{-1}$ LC50 for four *Unioidea* and one *Margaritiferidae* species in a 96h test; Wang et al. 2017). For juvenile *Utterbackia imbecilis*, Ni LC50 was $240\ \mu\text{g L}^{-1}$ in a 48h test (Keller and Zam 1991), and for juvenile *Hamiota perovalis* and *Villosa nebulosa*, the Ni EC50 values for a 96h test were $313\ \mu\text{g L}^{-1}$ and $510\ \mu\text{g L}^{-1}$, respectively (Gibson et al. 2018). Thus, despite the relatively short period of extremely high Ni exposure, the spill most likely had direct toxic impacts on mussels along the river. Mussels are known to detect harmful substances in the water and to escape short-term contamination by closing their shell (e.g. Naimo et al. 1992; Pynnönen and Huebner 1995), although this pollution avoidance mechanism is only temporary because of their metabolic needs (Cope et al. 2008). Douda (2010) reported that *A. anatina* is more sensitive than *U. crassus* to nitrate pollution, thus the variation in species-level sensitivity may explain the differences in mortality. Further, there is interesting species-level variation in the mussels ability to detect harmful elements; *A. anatina* did not exhibit shell closure during Hg exposure and was thus eliminated, whereas *Corbicula fluminea* did exhibit shell closure and was not harmed, even at the highest Hg concentration (Oliveira et al. 2015). Pynnönen (1990) reported the highest Ca^{2+} hemolymph concentrations for *U. tumidus* and *U. pictorum*, while it was lowest for *A. anatina*. One of the most important toxic mechanisms of Ni in freshwater animals is disruption of Ca^{2+} homeostasis (Brix et al. 2016), and Ca^{2+} is known to compete with metal ions by a variety of ways, such as blocking the entry of metals inside the cells (e.g. de Paiva Magalhaes et al. 2015). Thus, species-level differences in mussel biology may also explain the variation in Ni tolerance.

According to the OIVA Database (2019), River Kokemäenjoki surface water temperature in Harjavalta town was around 25°C in July–August 2014. Since this temperature was considerably high (average July–August temperature has been 19.2°C between 1961–2018, N 60; OIVA Database 2019), the mussels may have been negatively affected by elevated temperatures (Gagnon et al. 2014; Golladay et al. 2004; Haag and Warren 2008; Falfushynska et al. 2014). In River Rhine, *A. anatina*, *U. tumidus* and *U. pictorum* are restricted to sites where the water temperature does not exceed 24°C (*A. anatina*, *U. tumidus*) or 28°C (*U. pictorum*) (Verbrugge et al. 2012). Thus, even though it does not explain the mortality differences between species, the abnormally high temperature can be considered as an additional stressor during the spill. In addition to direct temperature stress, the high water temperature during the spill may have shortened the period of valve closure. Metabolic

needs (oxygen demand) increase in warm water, while oxygen solubility in warm water decreases (Galbraith et al 2012). Thus, the high temperature during the NiSO₄ spill may have intensified the exposure to pollution because of the resulting increased filtration volumes (Beggle et al. 2017) and shortened period of valve closure. Further, according to Brix et al. (2016), one of the toxic effects of Ni in aquatic biota is the impaired respiration, which may have had negative synergic effect with high temperature.

The differences in species recovery are difficult to untangle, but the differences could be related to higher spill-induced juvenile mortality among *A. anatina*, *U. crassus* and *U. pictorum* when compared to *U. tumidus*. Indeed, if the small individuals were lost from the population in 2014 spill, the population recovery would have been delayed. In addition, the decline of *U. crassus* density and relatively high recent mortality (when compared to other species) in 2017 below the spill site is particularly interesting. This observation means that the *U. crassus* population has been on the decline since 2014. The proportion of worn *U. crassus* (which indicates the mortality on longer term; Online resource 1) was not higher when compared to other species in 2014 which suggests that the *U. crassus* mortality was not elevated before the spill. Species specific “semi-chronic” mortality, which recurs over multiple years and phenomenon of delayed mortality, has been observed in many mussel die-off cases (Richard 2018). Higher mortality of *U. crassus* in 2017 seems not to be connected with tissue Ni concentrations (Online resource 3), because the highest concentrations in 2017 were measured in *A. anatina* samples.

Population size (or number of recently dead mussels) estimation is extremely difficult due to the lack of data regarding detection probability. Another issue is the spatial heterogeneity typical for mussels, which is reflected here as high variation in mussel density between sites. This is highlighted in reference sites, where the number of transects is low and the mussel density differences between 2014 and 2017 may be effected by transect placement in high-density sites. Nevertheless, crude numbers can be retrieved by simple extrapolation of mussel numbers. Despite the high uncertainty regarding these numbers, it is likely that as many as 16 million mussels were living in the impacted river section before the spill, and the magnitude of die-off could have been around 4 million mussels (1 million of *U. crassus*).

5. Conclusion

In conclusion, although our results clearly indicate that *A. anatina* was the most sensitive species to the NiSO₄ pollution plume (followed by *U. pictorum*, *U. crassus* and *U. tumidus*), the underlying mechanism of this increased sensitivity remains unsolved. It seems that *A. anatina* holds some potential for early warning species in northern boreal riverine systems. However, more research is clearly needed. Most urgently, the basic toxicological studies should be conducted for adult European unionid species. In addition, the species variation in their ability for prolonged shell shutdown is an important issue that should be clarified with European species. The role of elevated temperatures should also be studied, since the ability to keep the shell shut is

probably affected by temperature due to elevated metabolism and lower oxygen content in warm water. This is especially relevant in light of recent and future climate change. Moreover, the phenomenon of delayed mortality should be studied in order to understand the temporal aspect (and actual total impacts) of pollution incidents.

6. References

- Allen DC, Galbraith HS, Vaughn CC, Spooner DE (2013) A tale of two rivers: implications of water management practices for mussel biodiversity outcomes during drought. *Ambio* 42:881-891
- Anderson RM, Layzer JB, Gordon ME (1991) Recent catastrophic decline of mussels (Bivalvia:Unionidae) in the Little South Fork Cumberland River, Kentucky. *Brimleyana* 17:1-8
- Badra P (2011) Mussel shell survey report: Kalamanzoo River Unionid mussel shell survey in the Marshall and Battle Creek area October 2010. Michigan Natural Features Inventory.
- Beggel S, Hinzmann M, Machado J, Geist J (2017) Combined impact of acute exposure to ammonia and temperature on the freshwater mussel *Unio pictorum*. *Water* 9:455.
<https://doi.org/10.3390/w9070455>
- Brendelberger H, Klauke C (2009) Pedal feeding in freshwater unionid mussels: particle-size selectivity. *Int Ver Theor Angew Limnol Verh* 30:1082-1084
- Brix KV, Schlekot CE, Garman ER (2016) The mechanisms of nickel toxicity in aquatic environments: An adverse outcome pathway analysis. *Env Toxicol Chem* 36: 1128-1137
- Cope WG, Bringolf RB, Buchwalter DB, Newton TJ, Ingersoll CG, Wang N, Augspurger T, Dwyer FJ, Barnhart C, Neves RJ, Hammer E (2008) Differential exposure, duration and sensitivity of unionoidean bivalve life stages to environmental contaminants. *J N Am Benthol Soc* 27:451-462
- Crossman JS, Cairns Jr J, Kaesler RL (1973) Aquatic invertebrate recovery in the Clinch River following hazardous spills and floods. *Virginia Water Resource Center* 63:I-VII

398 de Paiva Magalhaes D, da Costa Marques MR, Baptista DF, Buss DF (2015) Metal bioavailability
399 and toxicity in freshwaters. *Environ Chem Lett* 13:69-87
400

401 Doua K (2010) Effects of nitrate nitrogen pollution on Central European unionid bivalves revealed
402 by distributional data and acute toxicity testing. *Aquat Conserv* 20:189-197
403

404 EEC (1992) Council Directive 92/43/EEC on May 1992 on the conservation of natural habitats and
405 of wild fauna and flora.
406

407 ELY (2019) Kokemäenjoen nikkelipäästö. Varsinais-Suomen ELY-keskus. Elinkeino-, liikenne- ja
408 ympäristökeskus. [http://www.ely-keskus.fi/web/ely/kokemaenjoen-](http://www.ely-keskus.fi/web/ely/kokemaenjoen-nikkelipaasto;jsessionid=1D1F4B2662F14F09C4EB9B365F0E47B4?p_p_id=122_INSTANCE_alu_evalinta&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&p_r_p_564233524_resetCur=true&p_r_p_564233524_categoryId=14406)
409 [nikkelipaasto;jsessionid=1D1F4B2662F14F09C4EB9B365F0E47B4?p_p_id=122_INSTANCE_alu](http://www.ely-keskus.fi/web/ely/kokemaenjoen-nikkelipaasto;jsessionid=1D1F4B2662F14F09C4EB9B365F0E47B4?p_p_id=122_INSTANCE_alu_evalinta&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&p_r_p_564233524_resetCur=true&p_r_p_564233524_categoryId=14406)
410 [evalinta&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&p_r_p_564233524_resetCur=true](http://www.ely-keskus.fi/web/ely/kokemaenjoen-nikkelipaasto;jsessionid=1D1F4B2662F14F09C4EB9B365F0E47B4?p_p_id=122_INSTANCE_alu_evalinta&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&p_r_p_564233524_resetCur=true&p_r_p_564233524_categoryId=14406)
411 [&p_r_p_564233524_categoryId=14406](http://www.ely-keskus.fi/web/ely/kokemaenjoen-nikkelipaasto;jsessionid=1D1F4B2662F14F09C4EB9B365F0E47B4?p_p_id=122_INSTANCE_alu_evalinta&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&p_r_p_564233524_resetCur=true&p_r_p_564233524_categoryId=14406). Accessed 20 May 2019
412

413 ESRI (2015) ArcGIS release 10.3.1. Environmental Systems Research Institute, ESRI. Redlands,
414 CA.
415

416 Esty D, Porter ME (2002) Ranking national environmental regulation and performance: A leading
417 indicator of future competitiveness? In: Porter ME, Sachs JD, Cornelius PK, McArthur JW, Schwab
418 K (eds) *The global competitiveness report 2001-2002*, Oxford University Press, pp 78-101
419

420 Falfushynska H, Gnatyshyna L, Yurchak I, Ivanina A, Stoliar O, Sokolova I (2014) Habitat
421 pollution and thermal regime modify molecular stress responses to elevated temperature in
422 freshwater mussels (*Anodonta anatina*: Unionidae). *Sci Total Environ* 500-501:339-350
423

424 Farris JL, Van Hassel H (2006) *Freshwater bivalve ecotoxicology*. CRS Press
425

426 Gagnon PM, Golladay SW, Michener WK, Freeman MC (2004) Drought responses of freshwater
427 mussels (Unionidae) in coastal plain tributaries of the Flint River basin, Georgia. *J Freshw Ecol*
428 19:667-679
429

430 Galbraith HS, Blakeslee CJ, Lellis WA (2012) Recent thermal history influences thermal tolerance
431 in freshwater mussel species (Bivalvia: Unionoida). *Freshw Sci* 31:83-92

432

433 Getis A, Ord J.K. (1992) The analysis of spatial association by use of distance statistics. *Geogr Anal*

434 24:189-206

435

436 Gibson KJ, Miller JM, Johnson PD, Stewart PM (2018) Acute toxicity of chloride, potassium

437 nickel, and zinc to federally threatened and petitioned mollusk species. *Southeast Nat* 17:239-256

438

439 Giger W (2009) The Rhine red, the fish dead – the 1986 Schweizerhalle disaster, a retrospect and

440 long-term impact assessment. *Environ Sci Pollut Res* 16:98-111. [https://doi.org/10.1007/s11356-](https://doi.org/10.1007/s11356-009-0156-y)

441 009-0156-y

442

443 Golladay SW, Gagnon P, Kearns M, Battle JM, Hicks DW (2004) Response of freshwater mussel

444 assemblages (Bivalvia: Unionidae) to a record drought in the Gulf Coastal Plain of southwest

445 Georgia. *J N Am Benthol Soc* 23:494-506

446

447 Gundacker C (2000) Comparison of heavy metal bioaccumulation in freshwater molluscs of urban

448 river habitats in Vienna. *Environ Pollut* 110:61-71

449

450 Haag WR, Warren ML (2008) Effects of severe drought on freshwater mussel assemblages. *Trans*

451 *Am Fish Soc* 137:1165-1178

452

453 Hammer Ø, Harper DAT, Ryan PD (2001) PAST. Paleontological statistics software package for

454 education and data analysis. *Palaeontol Electronica* 4(1):9

455

456 Havlik ME, Marking LL (1987) Effects of contaminants on Naiad mollusks (Unionidae): A review.

457 United States Department of the Interior Fish and Wildlife Service. Resource Publication 164,

458 Washington, D.C.

459

460 Inoue K, Stoeckl K, Geist J (2017) Joint species models reveal the effects of environment on

461 community assemblage of freshwater mussels and fishes in European rivers. *Diversity and*

462 *Distributions* 23: 284-296

463 Keller AE, Zam SG (1991) The acute toxicity of selected metals to freshwater mussel, *Anodonta*

464 *imbecilis*. *Environ Toxicol Chem* 10:539-546

465

466 Kryger J, Riisgård HU (1988) Filtration rate capacities in 6 species of European freshwater
 467 bivalves. *Oecologia* 77:34-38
 468
 469 KVVY (2015) 5.-6.7.2014 tapahtuneen nikkelpäästön vaikutusten selvittäminen/loppuraportti.
 470 Kirjenro 255/15. Kokemäenjoen Vesiensuojeluyhdistys ry.
 471
 472 KVVY (2016) 5.-6.7.2014 tapahtuneen nikkelpäästön vaikutusten selvittäminen / loppuraportti
 473 vuodelta 2015. Kirjenro 139/16. Kokemäenjoen Vesiensuojeluyhdistys ry.
 474
 475 Layzer JB, Madison LM (1995), Microhabitat use by freshwater mussels and recommendations for
 476 determining their instream flow needs. *Regul River* 10:329-345
 477
 478 Leppänen J, Weckström J, Korhola A (2017c) Multiple mining impacts induce widespread changes
 479 in ecosystem dynamics in a boreal lake. *Sci Rep* 7:1058
 480
 481 Lopes-Lima M, Kebapçı U, Van Damme D (2014) *Unio crassus*. The IUCN Red List of Threatened
 482 Species 2014: e.T22736A42465628. [http://dx.doi.org/10.2305/IUCN.UK.2014-](http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T22736A42465628.en)
 483 [1.RLTS.T22736A42465628.en](http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T22736A42465628.en)
 484
 485 Lopes-Lima M, Sousa R, Geist J, Aldridge DC, Araujo R, Bergengren J, et al. (2017) Conservation
 486 status of freshwater mussels in Europe: state of the art and future challenges. *Biol Rev* 92:572-607
 487
 488 Lopes-Lima M, Burlakova LE, Karatayev AY, Mehler K, Seddon M, Sousa R (2018) Conservation
 489 of freshwater bivalves at the global scale: diversity, threats and research needs. *Hydrobiologia*
 490 810:1-14
 491
 492 Lotsari E, Aaltonen J, Veijalainen N, Alho P, Käyhkö J (2013) Future fluvial erosion and
 493 sedimentation potential of cohesive sediments in a coastal river reach of SW Finland. *Hydrol*
 494 *Process* 28:6016-6037
 495
 496 Malmqvist B, Rundle S (2002) Threats to the running water ecosystems of the world. *Environ*
 497 *Conserv* 29:134-153
 498
 499 Mitchell A (2005) *The ESRI Guide to GIS Analysis, Volume 2*. ESRI Press

500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533

Naimo TJ, Atchison GJ, Holland-Bartels LE (1992) Sublethal effects of cadmium on physiological responses in the pocketbook mussel, *Lamsilis ventricosa*. Environ Toxicol Chem 11:1013-1021

Naimo TJ (1995) A review of the effects of heavy metals on freshwater mussels. Ecotoxicolgy 4:341-362

Nobles T, Zhang Y (2011) Biodiversity Loss in Freshwater Mussels: Importance, Threats, and Solutions. In: Grillo O (ed) Biodiversity Loss in a Changing Planet, InTech, Rijeka pp 137-162

OIVA Database (2019) The open database of the Finnish Environment Institute. Available at <https://www.syke.fi/en-US/Open_information> Visited 9.10.2019.

Oliveira P, Lopes-Lima M, Machado J, Guilhermino L (2015) Comparative sensitivity of European native (*Anodonta anatine*) and exotic (*Corbicula fluminea*) bivalves to mercury. Estuar Coast Shelf S 167:191-198

Pynnönen K (1990) Physiological responses to severe acid stress in four species of freshwater clams (Unionidae). Arch Environ Contam Toxicol 19:471-478

Pynnönen KS, Huebner J (1995) Effects of episodic low pH exposure on the valve movements of the freshwater bivalve *Anodonta cygnea* L. Water Res 29:2579-2582

Reck BK, Muller DB, Rostkowski K, Graedel TE (2008) Anthropogenic nickel cycle: Insights into use, trade and recycling. Environ Sci Technol 42:3394-3400

Richard J (2018) Freshwater mussel die-offs: insights from a compilation of known cases. Mollusk Health and Disease Workshop. Freshwater Mollusk Conservation Society, 13-15 March 2018, La Crosse, Wisconsin US.

Rzymiski P, Niedzielski P, Klimaszyk P, Poniedziałek B (2013) Bioaccumulation of selected metals in bivalves (Unionidae) and *Phragmites australis* inhabiting municipal water reservoir. Environ Monit Assess 186:3199-3212

534 Soldán P, Pavonič M, Bouček J, Kokeš J (2001) Baia Mare accident – Brief ecotoxicological report
 535 of Czech experts. *Ecotox Environ Safety* 49:255-261
 536

537 Stoeckl K, Geist J (2016) Hydrological and substrate requirements of the thick-shelled river mussel
 538 *Unio crassus* (PHILIPSSON 1788) *Aquatic Conservation: Marine and Freshwater Ecosystems* 26:
 539 456-469
 540
 541

542 Strayer DL (1999) Use of flow refuges by unionid mussels in rivers. *J N Am Benthol Soc* 18:468-
 543 476
 544

545 Verbrugge LNH, Schipper AM, Huijbregts MAJ, Van der Velde G, Leuven RSEW (2012) 14:1187.
 546 <https://doi.org/10.1007/s10530-011-0148-y>
 547

548 Vuori K-M, Mitikka S, Vuoristo H (2009) Pintavesien ekologisen tilan luokittelu.
 549 Ympäristöhallinnon ohjeita 3/2009. Suomen Ympäristökeskus, Sastamala
 550

551 Väisänen A (2018) Simpukoiden metallipitoisuudet Kokemäenjoella 2017. Raporttinro 573/18.
 552 KVVY Tutkimus Oy
 553

554 Vörösmarty CJ, McInture PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S,
 555 Bunn SE, Sullivan CA, Reidy Liermann C, Davies PM (2010) Global threats to human water
 556 security and river biodiversity. *Nature* 467:555-561
 557

558 Wang N, Ivey CD, Ingersoll CG, Brumbaugh WG, Alvarez D, Hammer EJ, Bauer CR, Augspurger
 559 T, Raimondo S, Barnhart MC (2017) Acute sensitivity of a broad range of freshwater mussels to
 560 chemicals with different modes of toxic action. *Environ Toxicol Chem* 36:786-796
 561

562 Watters GT (2006) A brief look at freshwater mussel (Unionacea) biology. In: Farris JL and Van
 563 Hassel H (eds) *Freshwater bivalve ecotoxicology*. CRS Press, pp 51-64
 564

565 Waverly AT, Cope WG, Shea D (2006) Toxicokinetics of environmental contaminants in
 566 freshwater bivalves. In: Farris JL and Van Hassel H (eds) Freshwater bivalve ecotoxicology, CRS
 567 Press, pp 169-213
 568
 569 Weber E (2005) Population size and structure of three mussel species (Bivalvia: Unionidae) in a
 570 northeastern German river with special regard to environmental factors. *Hydrobiologia* 537:169-183
 571
 572 Williams JD, Warren ML, Cummings KS, Harris JL, Neves RJ (1993) Conservation status of
 573 freshwater mussels of the United States and Canada. *Fisheries* 18:6-22
 574
 575 Zajac K, Zajac T, Cmiel A (2016) Spatial distribution and abundance of Unionidae mussels in a
 576 eutrophic floodplain lake. *Limnologica* 58:41-48
 577
 578 Woodfine DG, Havas M (1995) Pathways of chemical recovery in acidified, metal-contaminated
 579 lakes near Sudbury, Ontario, Canada. *Water Air Soil Poll* 85:797-803
 580
 581 Zuykov M, Pelletier E, Harper DAT (2013) Review: Bivalve mollusks in metal pollution studies:
 582 from bioaccumulation to biomonitoring. *Chemosphere* 93:201-208
 583
 584
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 586
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588 **Figure captions**

589
 590 **Fig. 1** a: Location of River Kokemäenjoki; b: River Kokemäenjoki catchment; c: Study area. Sampling sites
 591 are labeled according to their distance (m) from the Harjavalta hydropower plant reservoir dam. The spill site
 592 is indicated by the black arrow. The river flow direction is from SE to NW towards the Bothnian Sea. The
 593 red dot represents the approximate location of central Pori. Ulvila, Nakkila, Harjavalta and Kokemäki are
 594 small towns located by the river. Seaward, City of Pori and Small towns describe the general character of
 595 land area to the adjacent river section. Coordinates are in WGS84 system.
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598 **Fig. 2** Post-spill Ni concentration dynamics in River Kokemäenjoki. The values were obtained from ELY
599 (2019). The arrow indicates river flow direction (N = 28). Filled circles represent sampling sites.
600 Interpolation maps are created using spline with barrier -method in ArcMap 10.3.1 program (ESRI 2015).
601 River width has been edited for ease of interpretation.

602
603 **Fig. 3** Post-spill mussel mortality. White bars indicate pre-spill density (recently dead + alive mussels) and
604 black bars indicate the post-spill density (alive mussels). Bottom x-axis is for density data. The line indicates
605 the mortality percentage, upper x-axis is for percentage data. Gray areas represent statistically significant
606 mortality hotspots ($P < 0.01$).

607
608 **Fig. 4** Mussel post-spill mortality % at impacted sites. The box indicates 25–75 percent quartiles, whiskers
609 denote maximum and minimum values and horizontal line indicates median value.

610
611 **Fig. 5** 2017 mussel mortality. White bars indicate recent density (recently dead + alive mussels) and black
612 bars indicate the 2017 density (alive mussels). Bottom x-axis is for density data. The line indicates the
613 mortality percentage, upper x-axis is for percentage data. Note the scale is different for *U. pictorum*
614 percentage data (x-axis).

615