Annex C

Water quality and heavy metals in freshwater pearl mussels and their habitat

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1 Background

Each river is unique in respect to water qualities. In general, hydro-chemical conditions in rivers are determined by several environmental variables such as geological conditions, topology, vegetation, climatic conditions and anthropogenic activities will also differ as regards spatial scales – along the river, and time scales – within and between years.

In general, the chemistry of northern European waters varies considerably, although nutrient levels are generally low throughout the northern region of interest in this project e.g. due to low temperatures and corresponding relatively low biological activities. The rivers in northern Norway and the Kola Peninsula that flow on the Baltic Shield have generally low levels of dissolved solids (Brittain *et al.* 2009). Bedrock, soils and geography are very heterogeneous, resulting in a wide variation in natural water chemistry in the project area and also within the watercourses (Fig. 1). Each river with a freshwater pearl mussel population has its natural and unique chemical composition to which the mussel population has acclimatized. This must be taken into consideration when setting priorities for monitoring, whether it is to monitor the changes in the environment of freshwater pearl mussels in general or to reveal the parameters that require measures in order to restore mussel populations or to remediate their habitats. The main problem is then to understand what could be the human impacts and effect of these impacts together with the various other parameters influencing water quality in the individual rivers. Subsequently, it is also important to know whether any measures might result in side-effects or combination effects that are also harmful to the mussels. This is especially important in a long-term perspective.

The habitat changes may occur as a result of relatively modest climatic changes (Hastie et al 2003) and as well the physical microhabitat of the freshwater pearl mussel (Hastie et al 2000). Geist and Auerswald (2007) points out that the stream bed appears to be the most important habitat factor limiting the recruitment of freshwater pearl mussel, and that measures should be the long-term functioning restoration of natural flow and dynamic in the whole river catchment.

Human activities have become increasingly important to ecosystems also at high latitudes, and they impact them, for instance, through the long-range transport of pollutants and nutrients. In general they are impacted by climate change and locally by local sources such as settlements and urban areas, agriculture, reindeer husbandry, forestry, melioration. The two last named are the local factors with the greatest impact in the target area. Emissions and other effects of industrial activities on the Kola Peninsula as well as in Finland and Sweden are important factors in part of the target area, but also other long-distance transportation has an

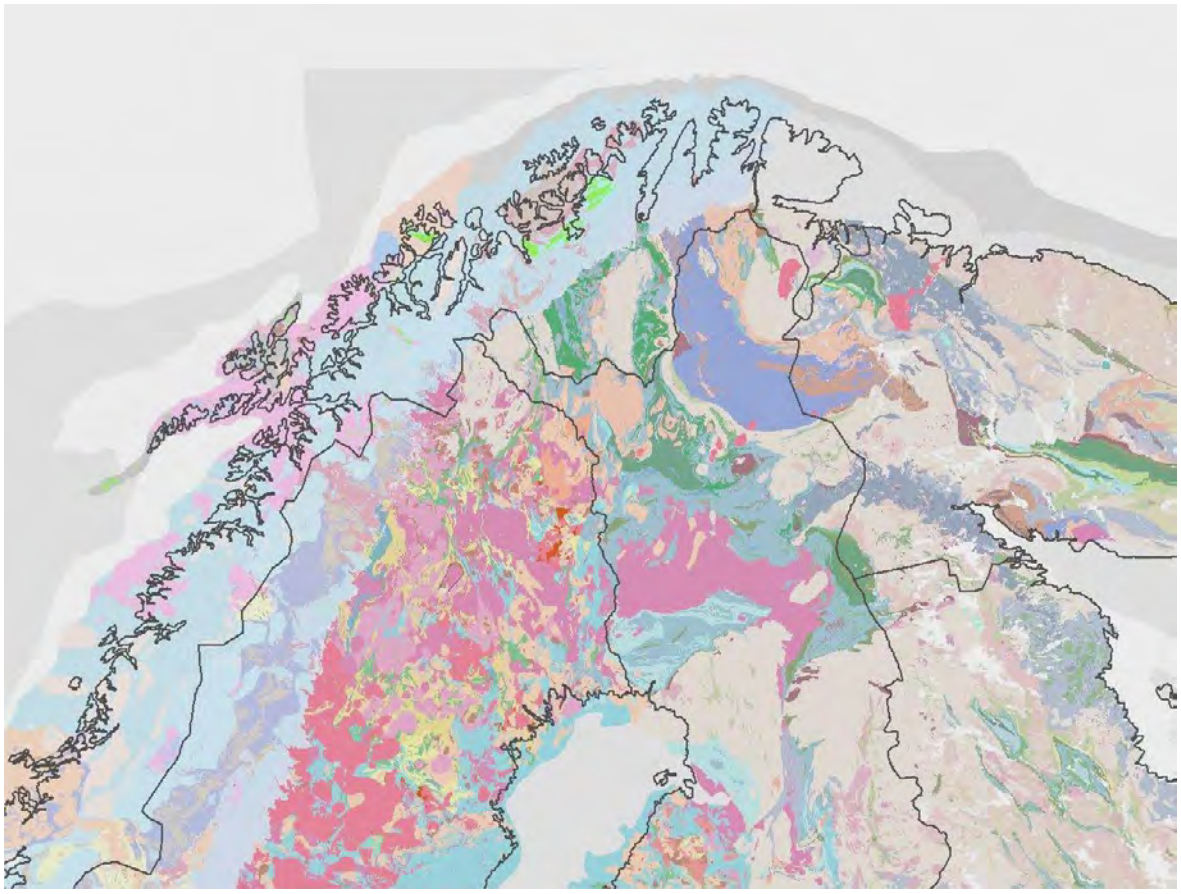


Figure 1. Bedrock map illustrating the great diversity in geological conditions in the region. Such high diversity also occurs at smaller spatial scales, (Norwegian Geological Survey). Map legends are given in Appendix 1.

influence. The type of soil in the catchments are then influencing the effects of all this factors, and is important also to know the type of soil and its physicochemical properties.

Threats to freshwater pearl mussels may be identifiable from existing information about the waters. This information is, however, relatively scarce and insufficient for conclusions to be drawn, and further investigations are, therefore, required. In several watercourses within our project area, regular or occasional water quality sampling have been carried out. These are often ordered by or conducted by the environmental authorities. However, only a few data are available for actual rivers containing freshwater pearl mussel populations. In order to increase the understanding of hydro-chemical processes in freshwater pearl mussel rivers, available water quality data from freshwater pearl mussel rivers in the target area with the best coverage of samples has been summarized and analysed in this project. In addition, within this project regular water sampling was carried out in four selected freshwater pearl mussel rivers in the target area.

Along with riverbed conditions and their substrate dynamics, governed by flow regime changes, a decline in water quality is often responsible for the loss of freshwater pearl mussel recruitment currently and in the past, and is probably the main cause of possible further extinction of populations (Larsen 1997). The juveniles are more sensitive to pollution than the adults, and persistent intermediate levels of eutrophication could prevent long-term recruitment, resulting in a population dynamics with ageing cohorts (Bauer 1988). The important parameters affecting recruitment are BOD (biochemical oxygen demand), and calcium and phosphate levels in the water. Increased content of total phosphorus and nitrogen, resulting in eutrophication since phosphate is usually the limiting factor for plant and algae growth, illustrates the importance of monitoring water qualities. According to data from Swedish rivers (Degerman *et al.* 2009) total phosphorus (totP),

concentration in rivers with freshwater pearl mussel populations varies from 5 to 15 µg/l during flooding. Degerman *et al.* (2009) suggest 10µg/l as a limit for freshwater pearl mussels. For Phosphate (PO_4) a concentration of 5 µg/l is suggested as a limit in Irish rivers (Moorkens 2006). One has to keep in mind that, during the vegetative period, all phosphate can be taken up by plants, so it is not found in free water. Bauer (1988) observed that adult mortality is correlated with nitrate concentration. A limit of 125 µg/l has been suggested for NO_3 (Moorkens 2006). Decomposing algae and other organic matter depletes oxygen and causes elevated total ammoniacal nitrogen (TAN) levels. pH in the rivers of the target-area are mostly too low to cause TAN to occur as ammonia (NH_4^+). Ammonia is not measured separately in water monitoring in Finland, in spite of its extremely poisonous effects on aquatic life. In addition, monitoring TAN fluctuations at different periods of the year is important, as this indicates human impact and the sediment situation in the river. NH_4-N oxidation causes low-oxygen conditions near the bottom and in sediment that is harmful to freshwater pearl mussels, especially for young individuals during the warm period. The end of summer and early autumn appear to be a critical period when TAN is high due to macroalgae (mainly filamentous algae) dying off while the water is not cold yet. Nitrification during winter is so slow that oxidation almost does not occur in shallow cooler sites. Nevertheless, it is going on in deeper warmer parts of the river system. In addition, during the summer intensive vegetation growth also expends oxygen and raises pH, resulting in TAN transforming to ammonia. Freshwater mussels are more sensitive to ammonia than many other benthic species (Wang *et al.* 2007).

Elevated turbidity and level of particles and suspended solids are among the biggest threats to freshwater pearl mussel populations, causing stress and clogging of the river beds. Average turbidity during spring flood should not exceed 1 FNU (Degerman *et al.* 2009), and a suspended solids level of <3 mg/l is suggested by Österling *et al.* (2010). It is also important that the humus content is not too high. This is often noted as water colour, and in Sweden, 80 mg Pt/l is considered to be the upper limit for freshwater pearl mussels (Degerman *et al.* 2009).

Conductivity is also connected to total dissolved solids as well as to dissolvents of salts. Good environments for freshwater pearl mussels usually have low conductivity. Freshwater pearl mussels prefer neutral or slightly alkaline water, and the lower pH limit in Scandinavia is suggested to be 6.2 (Degerman *et al.* 2009). Alkalinity is often low in the rivers in the target region, and water is acid-sensitive, and the freshwater pearl mussel prefers rivers that are not too low in calcium. Acid waters come in combination with certain chemicals and metals, often resulting in more toxic components of this chemical. Metal activity is also affected by the calcium concentration and the content of complex forming substances. Humus forms complexes with metals, reducing the proportion of ionic metals in the water, but it also, for instance, inhibits the oxidation of toxic ferrous iron to the less toxic ferric form (see Vuorinen *et al.* 1988, for references). Iron content varies a lot and is high in many Finnish rivers, but how iron affects the aquatic system also depends on temperature, light, flow condition, etc. Fe (II) oxidation can be accelerated by other metals, phosphates, fluoride, the abundance of the bacteria *Thiobacillus ferrooxidans*, and slowed down by e.g. sulphate, nitrate and chloride (Vuori 1995). The complex processes, shifting elemental compositions and ecological effects, make it hard to set any clear limit values. Linton *et al.* (2007) do, however, suggest 210 µg/l of total iron as an upper limit for sensitive benthos. Iron content is often higher in Finnish and Swedish rivers, and an iron peak of 500 µg/l was shown not to be harmful to the freshwater pearl mussel at normal pH (Taskinen *et al.* 2011), but in combination with low pH conditions the cumulative effect is likely to affect recruitment. Aluminium can also be high in rivers in the target area. During acidic events, the content of poisonous inorganic aluminium increases substantially. Inorganic aluminium is not normally measured in Finland. Total aluminium is measured mostly during winter. However, very few data series are available from snow-melt (spring series) and flood periods, when pH can be dangerously low when conversion to harmful aluminium may become abundant. In some water acidification

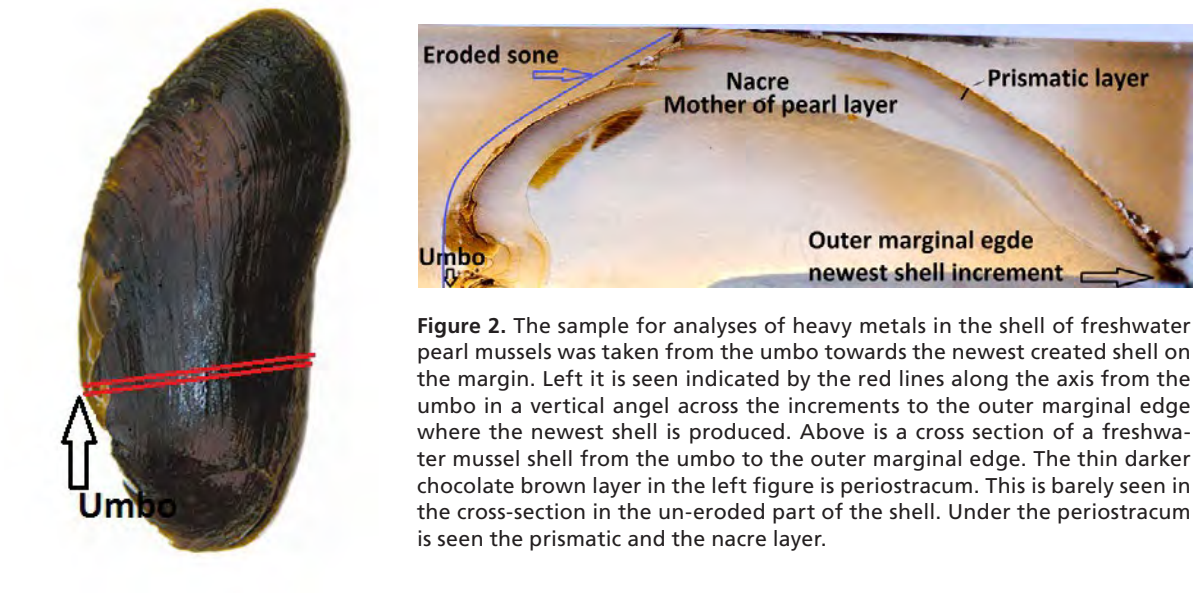


Figure 2. The sample for analyses of heavy metals in the shell of freshwater pearl mussels was taken from the umbo towards the newest created shell on the margin. Left it is seen indicated by the red lines along the axis from the umbo in a vertical angel across the increments to the outer marginal edge where the newest shell is produced. Above is a cross section of a freshwater mussel shell from the umbo to the outer marginal edge. The thin darker chocolate brown layer in the left figure is periostracum. This is barely seen in the cross-section in the un-eroded part of the shell. Under the periostracum is seen the prismatic and the nacre layer.

studies, inorganic aluminium in the Vätsäri area acid lakes was < 20 µg/l (Puro-Tahvanainen & Luokkanen 2007). Inorganic aluminium for freshwater pearl mussels should be less than 30 µg/l (Degerman *et al.* 2009).

The main component in the shell of freshwater pearl mussels is calcium (Ca) as a part of calcium carbonate ($CaCO_3$). The Ca content in the shell reflects the Ca availability in water, but is also influenced, for instance, by genetic attributes and is governed by the metabolic activity of the mussels. This may be influenced by temperature and the physical and chemical properties of the water through the growing season of the mussel. Animals with calcareous hard parts such as brachiopods and bivalve molluscs living under reduced oxygen and lowered pH values remain thin-shelled and small and grow very slowly (Rhoads & Morse 1971). The pollutants can affect the building of the shell, as in high concentrations it may impede the calcification processes, by reducing of the energy available to the mussel, since the specimen needs to allocate more energy to detoxification or metabolic functions. Species of the genus Unio has been demonstrated as making significant reductions in shell growth in response to oxygen depletion and elevated levels of phosphorous and organic carbon (Mutvei *et al.* 1996).

In the study by Dunca *et al.* (2005), it is demonstrated that increasing acidification in Swedish freshwater pearl mussel rivers has resulted in diminished growth rates. Mitigation of the negative effects of low pH on the ecosystem stability was accomplished by liming during the mid and late 1970s and since the early 1980s, after which pH returned to normal levels and shells grew much faster than during times of environmental stress (Dunca *et al.* 2005).

Also, the quality of the shell calcic microstructures in the various layers (prismatic and nacre layer) can be negatively influenced by pollutants. In freshwater pearl mussels, the oldest part of the shell is named the umbo area (see Fig. 2). In this area the erosion starts mainly caused by several factors including shell-eating bacteria. The shell to become eroded through the periostracum, the brown surface layer, and further through the prismatic (calcium) layer into the nacre (mother of pearl) layer after some decades. As the mussel grows older, the eroded part expands. Then there is difference between age groups of the mussels and therefore there will be a variation in the shell both between and within populations from natural causes. However, the variation in a population may indicate the shell-building activity.

2 Material and methods

2.1 Water quality

Threats to freshwater pearl mussels may be identifiable from existing information about the water. But this information is not sufficient for conclusions to be made, and further investiga-

tions are required. Regular or random water quality monitoring, conducted by the environmental authorities, is performed in quite a number of watercourses in the project area, but only a few data are available for freshwater pearl mussel rivers. For a better understanding of the hydro-chemical processes in freshwater pearl mussel rivers, available water quality data from rivers containing freshwater pearl mussel populations in the target area was summarized and analysed. Data from regular water sampling was collected within this project in four freshwater pearl mussel rivers of the target area; the Lutto, Näätämö/Neiden, Karasjohka and Bergmyrbäcken (Fig. 3).

Water quality information from the target area rivers was gathered and analysed from Finnish (Finnish Environmental Database Hertta), Norwegian (NIVA and NINA) and Swedish (Swedish University of Agricultural Sciences, Institutionen för vatten och miljö) authorities and institutes for the long-term studies and the spring series. Some of these data are regularly sampled while others are more scattered and sporadic.

Näätämö/Neiden

Sulphur emissions from Kola smelters have caused small lake acidification in an eastern part of the Näätämö watercourse (Kähkönen 1996, Lappalainen *et al.* 1995), where there is a high proportion of exposed bedrock, so the buffering capacity of the soil is low for miner-

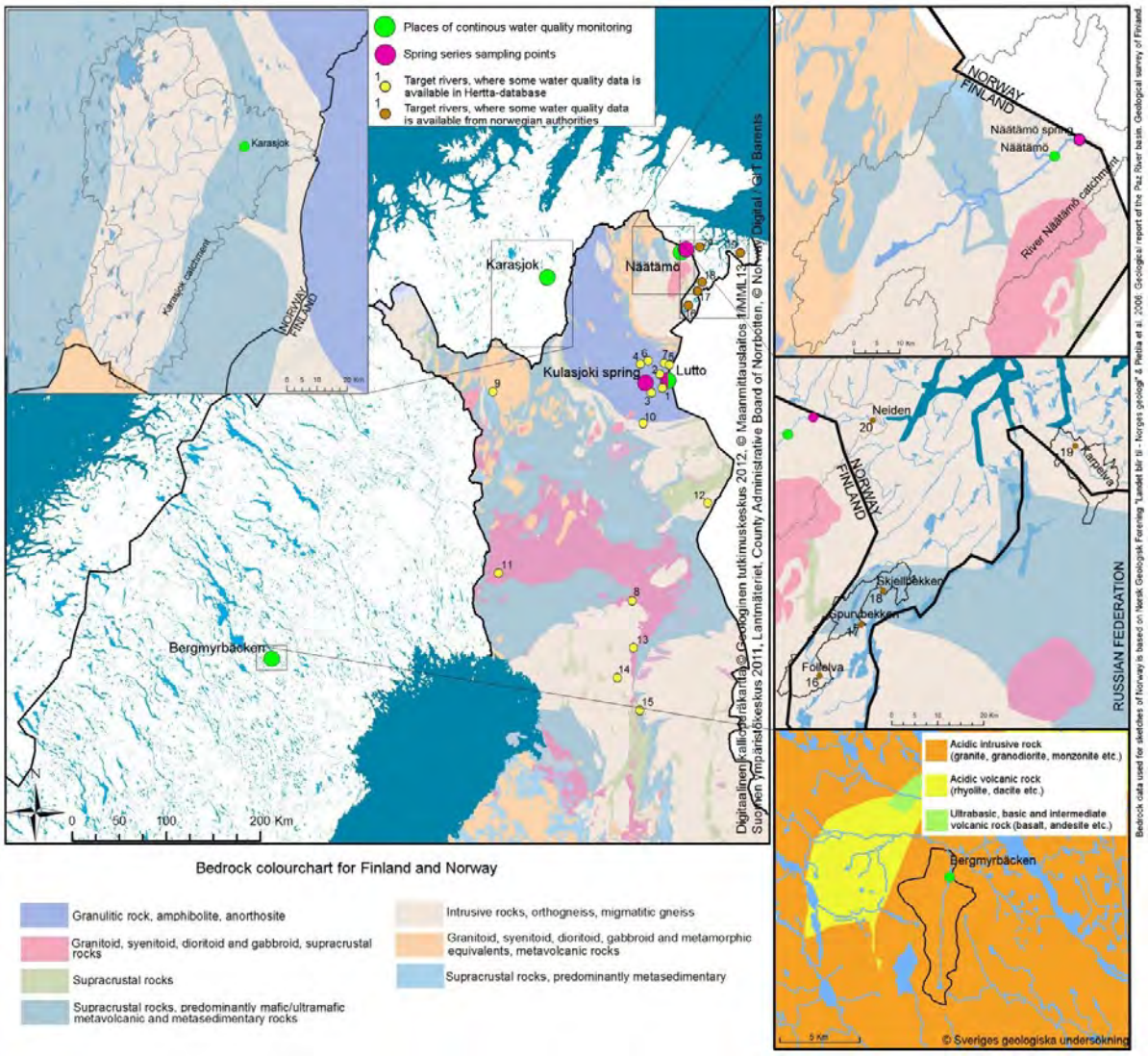
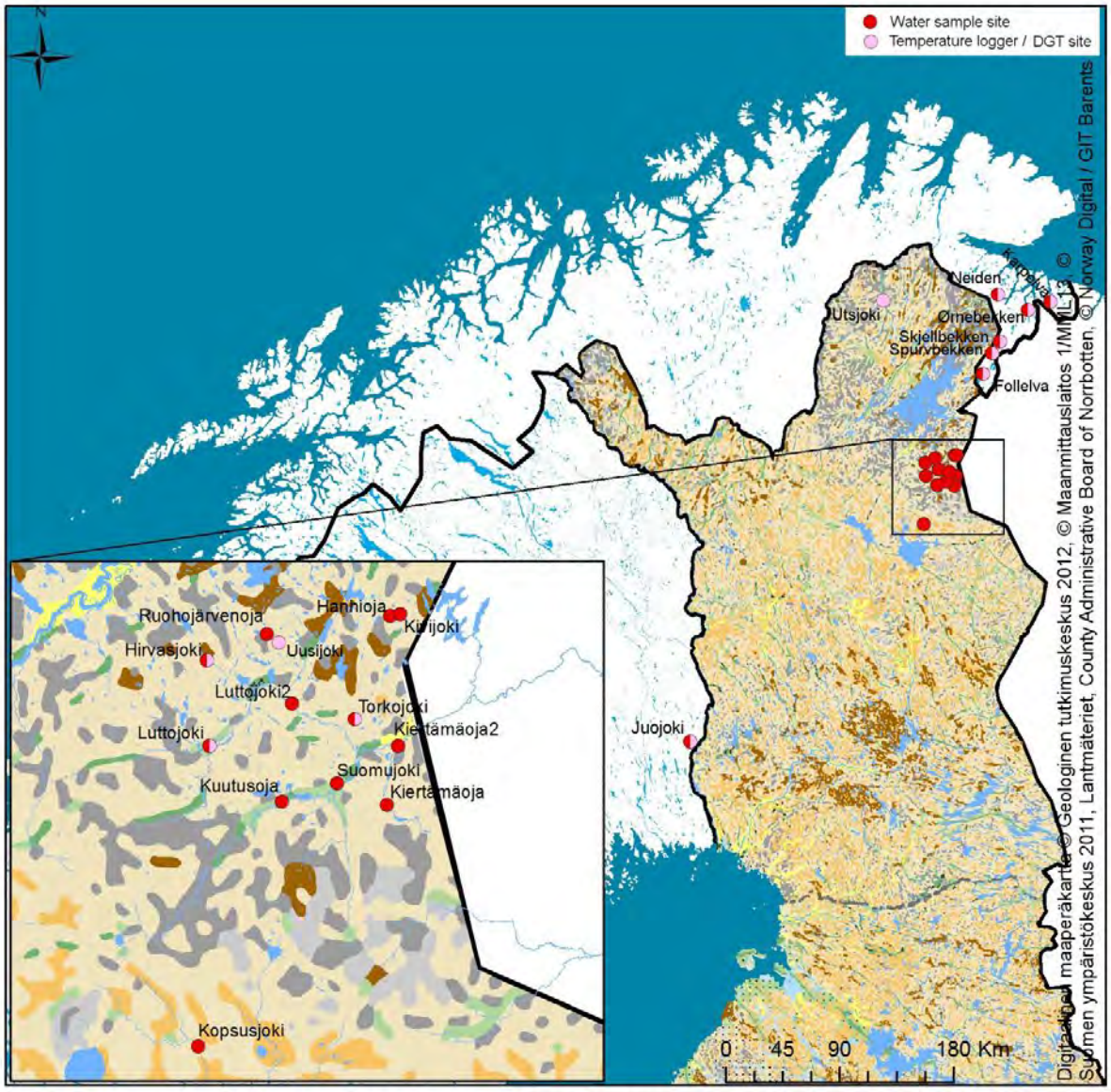


Figure 3. Map of the target area in northern Sweden, Finland and Norway, with the investigated freshwater pearl mussel rivers. Water quality data available for target rivers from environmental databases and bedrock geology of sampled areas are indicated. © Metsähallitus 2015, © Geological Survey of Finland 2015, © SYKE 2015, © National Land Survey of Finland 1/MML/15, © Läntmäriet, County Administrative Board of Norrbotten, © Norway Digital / GIT Barents.



COLOUR CHART FOR SURFACE DEPOSITS

- Outcrops of Pre-Quaternary bedrock
- Eluvial boulder field
- Gravelly and sandy till
- Silty till
- Hummocky moraine
- Esker, delta, sandur, sorted ice-marginal deposit
- Interlobate esker
- Till-covered esker / other till-covered gravel and sand deposit
- Gravel and sand deposit outside eskers
- Littoral gravel and sand deposit
- Fluvial deposit
- Homogeneous clay and silt deposit
- Varved clay and silt deposit
- Peat deposit
- Water

Figure 4. Map of the target area in northern Sweden, Finland and Norway, where soil and surface deposits of sampled areas are indicated as well as water samples, temperature loggers and DGT (passive sampler of heavy metal in water). © Metsähallitus 2015, © Geological Survey of Finland 2015, © SYKE 2015, © National Land Survey of Finland 1/MML/15, © Läntmäriet, County Administrative Board of Norrbotten, © Norway Digital / GIT Barents.

alogical reasons and because the soil is thin or completely lacking (Pietilä *et al.* 2006, see Fig. 3 and Fig. 4 for soil). Small lakes and watercourses are also more sensitive to acidification than big ones (Mannio 2001). According to Pietilä *et al.* (2006), pH values of below 6 occurred at the sampling points on the Finnish side in the vicinity of the granite area up to the felsic gneiss area near Näätämö (granitoids and intrusive rocks, Fig. 3). Depending on composition, these rocks can release potassium, aluminium and iron into the soil. These rock types have a reducing effect on the amounts of base ca-ions in the soil, and therefore on the buffering capacity (Pietilä *et al.* 2006). However, sulphur emissions from Kola industrial centres have been reduced over recent decades, resulting in noticeable chemical and biological recovery in this area (Tammi *et al.* 2003) Some other pollutant concentrations such as Cu and Ni are still high near the Finnish border (Puro-Tahvanainen & Luokkanen 2007), but do not exceed Swedish water quality criteria, according to what harmful biological effects may occur in sensitive waters if the Cu concentration exceeds 3 µg/l or the Ni concentration exceeds 15 µg/l (Alm *et. al.* 1999).

Mafic/ultramafic meta-volcanic and meta-sedimentary rocks influence the middle part of Näätämö course, where also freshwater pearl mussel population have historically been most abundant. These are alkaline rocks, containing large amounts of iron and easily soluble base cations, especially magnesium and calcium. But the proportion of heavy metals such as chromium and nickel in alkaline rocks is also relatively high (Pietilä *et al.* 2006).

Lutto

The Lutto catchment area in Finland lies completely on a granuline belt. Depending on the mineral composition, soluble aluminium or iron, or base cations, heavy metals and sulphur, may occur in the soil, but also large amounts of easily soluble base cations, especially calcium (Pietilä *et al.* 2006). Bedrock is partly covered with moraine, gravelly and sandy till (Fig. 4). General conductivity and alkalinity in the catchment are still low and acidification is observed in some studied sites of the Lutto catchment (Puro-Tahvanainen & Luokkanen 2007).

Karasjohka

The Karasjohka River lies partly on an alkaline greenstone belt and partly on area of acidic gneisses (Braathen & Davidsen 2000). Greenstone is an alkaline rock type, which can be rich in Cu and arsenic (Tarviainen *et al.* 1995), but very many different rock types are influencing in the area referred as greenstone belt on a generalized geological map.

Bergmyrbäcken

This river lies mostly on granites and granitoids low in base cations, which makes this area sensitive to acidification.

Other rivers

In the northern part of the target area soil is thinner, so bedrock has somewhat more influence on water chemistry than in the southern part of target area. Even though sample sites in the southern part are also situated on base cation-low granite and gneiss bedrock, there are more peat deposits in the southern part of the area affecting water quality. In the northern part the dominant glacial sediment type is till. The mineralogical composition of till mainly reflects the local bedrock. Most of the sampled Norwegian rivers run through a gneiss area, except Skjellbekken and Spurvbekken, These two rivers are in the area of basaltic vulcanite, part of the greenstone zone, extending into Norway from Russia. The map is generalized; actually there is considerable geochemical and mineralogical variation of the bedrock at the local level, affecting the chemical properties of the soil as well (Pietilä *et al.* 2006).

2.2 Regular monitoring of water quality

Regular water quality monitoring is going on in four fresh water pearl mussel rivers of the target area. Data from the entire sampling period was analysed for trends and seasonal fluctuations and to reveal possible reasons why all these rivers hold only aged freshwater pearl mussel populations. Water quality monitoring stations are: River Näätämö – sampled since 1980 (III, V, VIII, X); River Lutto – sampled

Table 1. Amount and sampling time of sparse water quality data on target freshwater pearl mussel rivers available in Hertta. Numbers refers to the numbers on the map in Fig. 3.

No	River	Samples	Period
1	Kiertämäjoki	n=2	1992–1993
2	Torkonjoki	n=4	1991–1992
3	Suomujoki	n=5	1990–2012
4	Hirvasjoki	n=2	1992
5	Hanhioja	n=1	1992
6	Uusijoki	n=1	1992–1993
7	Kivijoki	n=1	1992
8	Siikajoki	n=96	1987–2011
9	Onnasjoki	n=1	1988
10	Kopsusjoki	n=9	1972–1980
11	Koutusjoki	n=14	1978–2010
12	Saukko-oja	n=15	1980–2002
13, 14	Livojoki	n=556 (90 used)	1968–2012
15	Haukioja	n=1	1994
16	Föllelva	n=1	2012
17	Spurvbekken	n=11	1997–2003
18	Skjellbekken	n=16	1997–2012
19	Karpelva	n=10	2003–2012
20	Neiden	n=16	1990–2012

Figure 5. A DGT is seen operating in the current of a freshwater mussel river while it is anchored in a plastic mesh attached to river stones. Photo Paul Aspholm.

since 1992 (III, V, VIII, X); River Karasjohka – sampled since 1989, monthly since 1992; River Bergmyrbäcken – sampled since 1995, monthly since 2008. Everyday sampling data during the spring flood (spring series) were available for the Näätämö (1986, 1989), Lutto (1989) and for the Kulasjoki, which is a tributary of the River Lutto– (1989). Sparse water quality data were found for 15 more target freshwater pearl mussel rivers (Table 1), and thus some conclusions were possible for only five rivers, where five or more samples (together with our sample in this project in 2013) over the last 20 years were found.

Regular water sampling in the Rivers Lutto and Näätämö in Finland is performed four times per year by the Finnish environmental authorities (monitoring began in the 1980s on the Näätämö and in the 1990s on the Lutto); the Karasjok in Norway and Bergmyrbäcken in Sweden are sampled every month, but regular sampling started later. Unfortunately, all these rivers hold only aged freshwater pearl mussel populations with very little or no recruitment and water quality sampling stations are mostly a way downstream from the main freshwater pearl mussel area, so they are not very suitable for freshwater pearl mussel monitoring.

2.3 Series from temperature loggers

The use of loggers provides large datasets of continuous records of temperature. When records are made half-hourly or hourly, there is the possibility to see detailed changes in temperature. Multiple records make it possible to find temperature peaks and calculate temperature-sum and degree-days. This enables us to understand metabolic processes better, like turnover rates, energetic demand, various productions like gonad development and glochidia development in mothers and host.

2.4 Heavy metals in the water

The **d**iffusive **g**radient in **t**hin film sampler (DGT), is a passive sampling device that accumulates chemicals continuously from the water and can provide a **t**ime **w**eighted **a**verage (TWA) concentration of pollutants over the exposure period when the sampler is submerged and sampling (Fig. 5). Hence, they may offer a

number of advantages over other conventional monitoring techniques such as spot or grab sampling. However, little is known in detail of how such samplers respond to fluctuating concentrations, that is what happens under the spring and autumn flood. The use of DGT with open pore gels allows the labile fraction of metal associated with large organic ligands, or dissolved organic carbon (DOC) to be differentiated and quantified. However, the DGT gives a good indication of how the metals are accumulated during the period they are applied in the river. However, the results from the DGTs are not directly comparable to results from water samples collected in bottles during regular sampling.

The DGTs in this project were placed out in the rivers just prior to the snow-melt in May and replaced by new ones at the end of the spring flood period at the end of June. These first DGTs are called spring samplers. The second samplers were gathered in September. In Karpelva, two sites were used, the upper site at a location above agricultural influence where there are freshwater pearl mussels present and a site further down close to the outlet of the river. On some occasions, the DGT was lost during the flood or removed by other people, which is why is only one record from Ørnebekken (spring), Utsjoki (spring) and Juojoki (summer). The samples were analysed at the Norwegian Institute for Water Research.

2.5 Heavy metals in the sediment

Sediment samples were collected from sites where groups of freshwater pearl mussels were found to be living, except in River Neiden where only a few mussels were found very sparsely. The sample was taken in the substrate within the occurrence of the group of mussels. Two types of habitats were selected; one with young (less than 20 mm) and adult individuals and the other at sites where only adults are present. The distance between these two sites was 20–50 m. Samples were collected with a core cylinder (diameter 50 mm) down to 5 cm depth of the sediment, i.e. the depth of occurrence of the young mussels. The samples were analysed for heavy metals at the INEP lab in Apatity, Russia by the use of MS-ICP and data expressed as µg/g dry weight.

2.6 Heavy metals in the freshwater pearl mussel shells

Almost 30 heavy metals and nutrients were analysed in freshwater pearl mussel shells from 131 individuals from six rivers. The samples from Rivers Skjellbekken, Spurvbekken, Føllelva (in Norway) and Juojoki (in Sweden) consist of 30 shells from each river. These are the same individuals that have been analysed for heavy metals in the foot (soft tissue; see previous section) and for the genetic analyses described in Chapter 4. In addition, there are also samples of 10 shells from Ljusträskbäcken (in Sweden): shells that were collected as recent naturally dead mussels in the river. From the Lutto catchment in Finland one shell from a dead mussel has been analysed; and an individual collected from a heap of shells, from mussels killed by pearl fishers more than 50 years ago. However, it was well preserved and not lying in direct contact to the soil.

The main component in the shell of freshwater pearl mussels is calcium (Ca) as a part of calcium carbonate ($CaCO_3$). The Ca content in the shell reflects the Ca availability in water, but is also influenced, for instance, by genetic attributes and governed by the metabolic activity of the mussels. This may be influenced by temperature and the physical and chemical properties of the water through the growing season of the mussel. The pollutants can affect the building of the shell as in high concentrations it may impede the calcification process by reducing the available energy of the mussel, since the specimen needs to allocate more energy to detoxification or metabolic functions.

Also, the quality of the shell calcic microstructures in the various layers (prismatic and nacre layer) can be negatively influenced by pollutants. In freshwater pearl mussels after some decades the oldest part of the shell – **the umbo area** – starts to become eroded through the **peristracum** – the brown surface layer – and further through **the prismatic layer** to **the nacre layer**. As the mussel grows older, the eroded part expands. Then there is difference between age groups of the mussels and therefore there will be a variation in the shell both between and within populations from natural causes. However, the variation in a population may indicate the shell building activity.

For the analyses of the heavy metals, a slice of the right shell of each individual from the umbo (old origin) to the outer (today's) margin of the shell was collected. Each shell slice was homogenized and a sample of the bulk of about 100 mg was taken for analysis, which was done using a High Resolution Inductive Coupled Plasma, (HR-ICP-MS) ELEMENT 2 (Thermo Electronics at NTNU Trondheim, Norway). All the results from this analysis are given as µg/g dry weight of shell.

2.7 Heavy metals in the soft tissues and shell of freshwater pearl mussels

Heavy metals enter the mussel body either as directly ingested or by absorption through gills, mantle or other body surfaces. The metals have different rates of absorption, storage or accumulation in the mussel body. Mussels themselves have various mechanisms to limit or eliminate unwanted metals. It is, however, important to remember that several metals such as zinc and copper are essential in various metabolic processes, and also that other heavy metals can be present without being harmful or negative to the organism.

If exposed to heavy metals above tolerance limits, the mussel organism can start metabolic or other processes to actively expel the metal. This may be, for example, via metallothioneins or biotransformation, where molecules are produced to transport the unwanted metals out of the organism. One such biotransformation is the embedding of metals in the shell, where it becomes bound to calcium carbonate.

Freshwater pearl mussels may reach very great ages of 200 years or more, and the metals in the shells therefore represent an accumulation over this very long time period. It should be noted that the accumulation in the soft tissue of the mussel is influenced by relatively new exposures and processes. The effect of substantial exposure to heavy metals is thus first tracked in various parts of the soft tissue, while in the longer run also it is found in the shells, which reflect exposure levels throughout the long lifetime of the mussel. The turnover in soft tissue may be about one to two decades.

The mussels collected from Rivers Karpelva (Kelv), Skjellbekken (SkB), Spurvbekken (SpB) Føllelv (Felv) in Norway and Juojoki (Juo) in Sweden were all old adult mussels ranging in age from about 70 to 300 years. Thirty individuals were collected from each river, and the same individuals were analysed for the content of heavy metals in their soft tissue and shell, and these same individuals were also used for the genetic analyses described in Annex D. In this work package, we have analysed the heavy metal concentrations in the foot of the mussels and in the shell (see Chapter 3.3.5).

3 Results

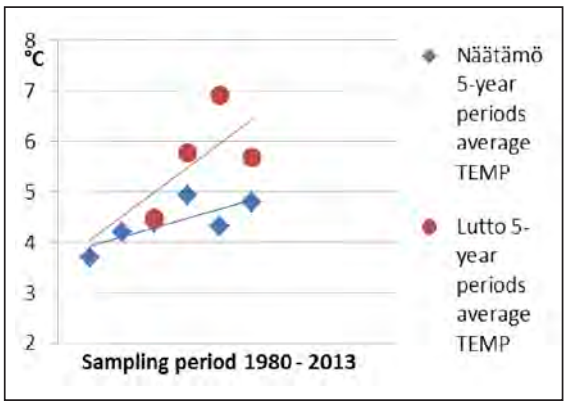
3.1 Regular monitoring

3.1.1 Median temperatures

Comparison of regular water quality data shows that annual temperatures do not differ a great deal, being highest in River Lutto (median 2.8°C, max 18.5°C) and lowest in the Karasjok (median 0.8°C, max 19°C), where fluctuations are also biggest (Fig. 6). Temperature shows a rising trend over the years in the Näätämö and Lutto, where the longest monitoring series are available (Fig. 6). Less clear results were obtained from the Karasjohka and Bergmyrbäcken, but they are pointing to the same development (Fig. 7). Still, the influence of forestry cannot be excluded; the most frequent clear-cut areas among these rivers are found near the Lutto, where the temperature rise is sharpest. This might indicate that it is the local climatic factors that influence together with more large-scale parameters. The smallest fluctuations are observed in Bergmyrbäcken, but the monitoring period there was short (2005–2013).

3.1.2 Nutrients

Total nitrogen is lowest and gives least peaks in the Lutto (median 100 µgN/l), in other rivers the median is around 200 µgN/l with occasional inputs up to 1,000 µgN/l in Näätämö and Karasjohka(Figs 8a–b). Also the worst nitrate (max 140 µgN/l, Figs 9a–b) and ammoniacal nitrogen (max 200 µgN/l, Figs 10a–b) peaks are observed in the Karasjohka. In the Lutto nitrate and total ammoniacal nitrogen (TAN) peaks are also low (Figs 8a–b), while even the frequency of TAN peaks is rising.



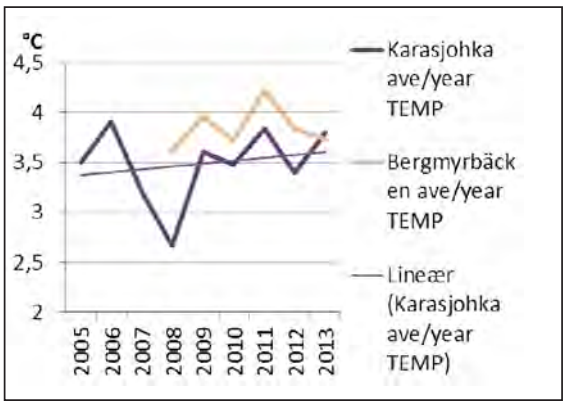


Figure 6. Temperature trends in the Näätämö, Lutto, Karasjohka and Bergmyrbäcken.

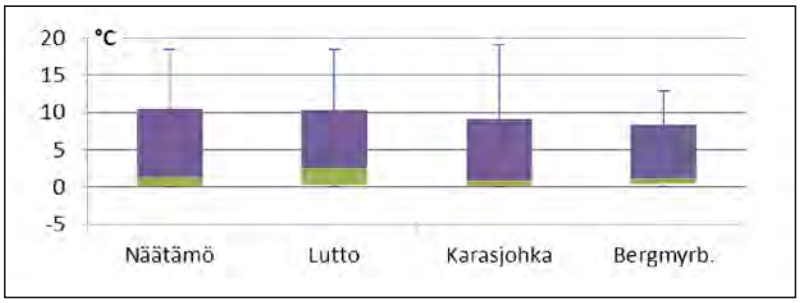


Figure 7. Median temperatures and temperature fluctuations in monitored rivers. In this and all similar plots below; the green (lower part of box) indicates a 25% level of samples, while the violet box indicate a 75% level, while the line between the green and violet indicates the median. The bars indicate minimum and maximum values.

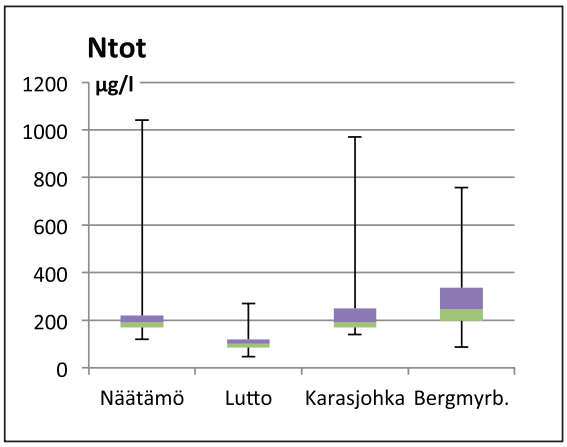


Figure 8a. Median total nitrogen, fluctuations in monitored rivers.

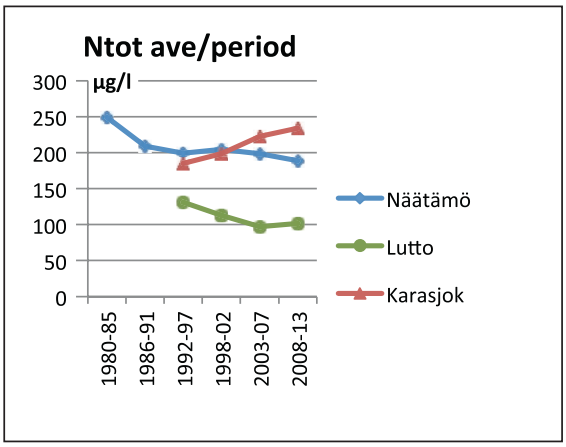


Figure 8b. Total nitrogen trends (averages of 5-year periods) in monitored rivers.

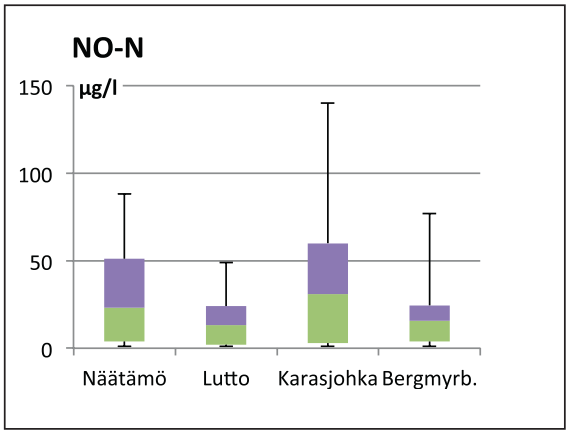


Figure 9a. Median nitrite + nitrate and its fluctuations in monitored rivers.

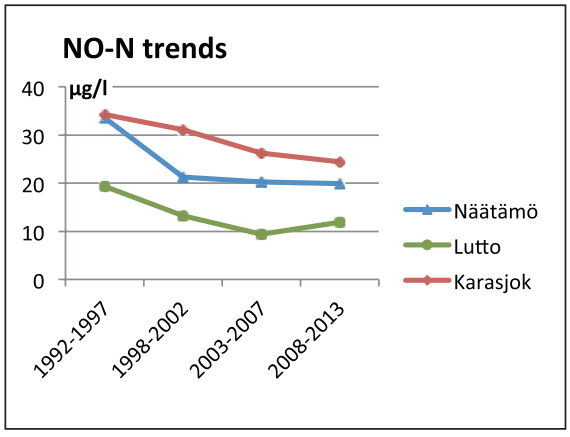


Figure 9b. Nitrate + nitrite trends from last 20 years, 5-year periods averages.

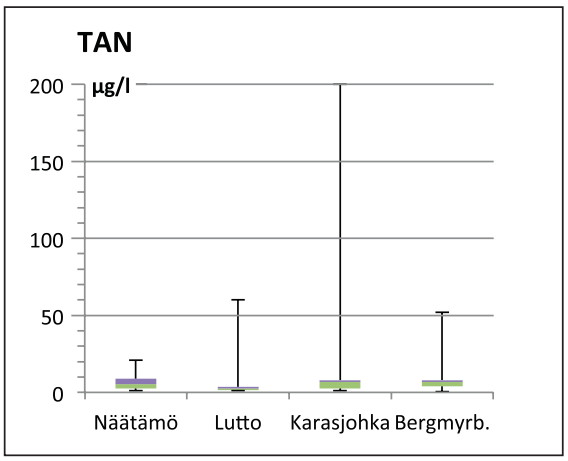


Figure 10a. Total ammoniacal nitrogen (TAN), median and fluctuations in monitored rivers.

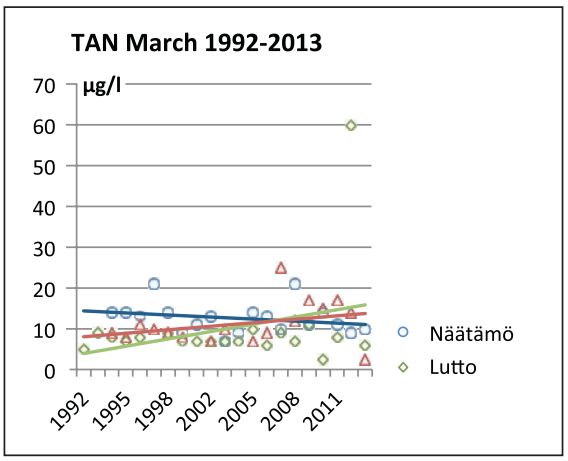


Figure 10b. (right) TAN trend lines from last 20 years, values of maximum season.

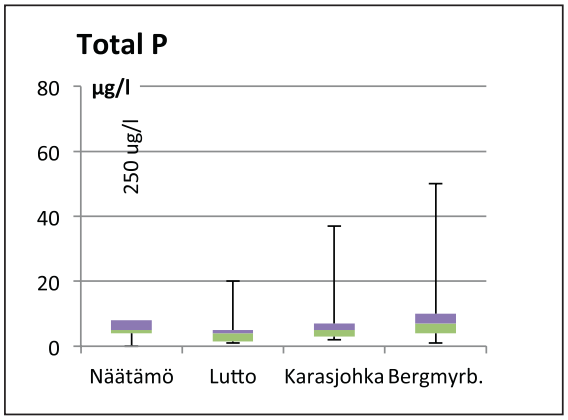


Figure 11a. Median total phosphorus and its fluctuations.

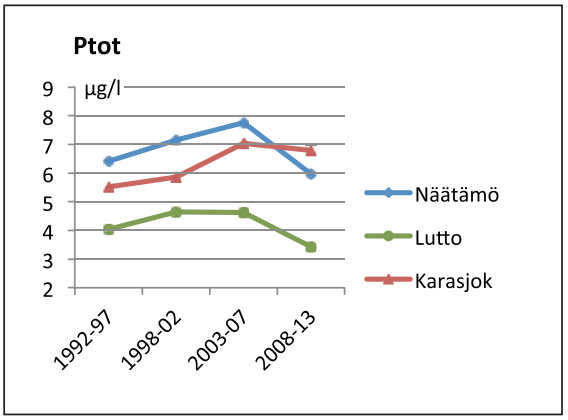


Figure 11b. Total phosphorus trends in monitored freshwater pearl mussel rivers.

Total nitrogen input has a clear rising trend over the last 20 years in the Karasjohka (Fig. 8b), while nitrate seems to have a declining trend (Fig. 9b). In the Lutto and Näätämö, sampling is too sparse to be give clear evidence; though nitrogen seems to have a declining trend there too. Intensified photosynthesis due to rising temperature can be the explanation. The rising trend in TAN (Total Ammoniacal Nitrogen) the Lutto and Karasjohka may be caused by decomposing algae, although sediment input pick up could also be due to forestry, reindeer husbandry, long-distance transportation or other reasons. The TAN is rising most quickly in River Lutto. Figure 10a shows TAN maximum season values and their trend lines (Fig. 10b) from the last twenty years. According to monthly sampling in the Karasjohka , the TAN maximum there is a little earlier than in March, but in the Näätämö and Lutto the first sample of year is taken in March and shows annual maximum values. In Bergmyrbäcken, the nutrients input is reduced after waterworks were built 2007.

In the rivers, compared total phosphorus is around 5 µg/l and PO_4 normally not detectable, but Ptot gives the highest peaks in the Näätämö (max 250 µg/l) and Bergmyrbäcken (Fig. 11a). The highest PO_4 peak, 13 µg/l is measured in the Lutto, (Fig. 10). Median PO_4 exceeds the detection limit only in Bergmyrbäcken (3 µg/l), but the situation is slightly improved there in recent years (Fig. 12).

3.1.3 Some other parameters

Dissolved (soluble) oxygen content in water should be at least 90 mg/l for freshwater pearl mussels. From regulatory monitored freshwater pearl mussel rivers in the target area dissolved oxygen (DO_2) in measured only in the Näätämö and Lutto. Figure 13 shows that oxygen content in both rivers is lowest in summer (August), not in winter under ice cover (March). Samples

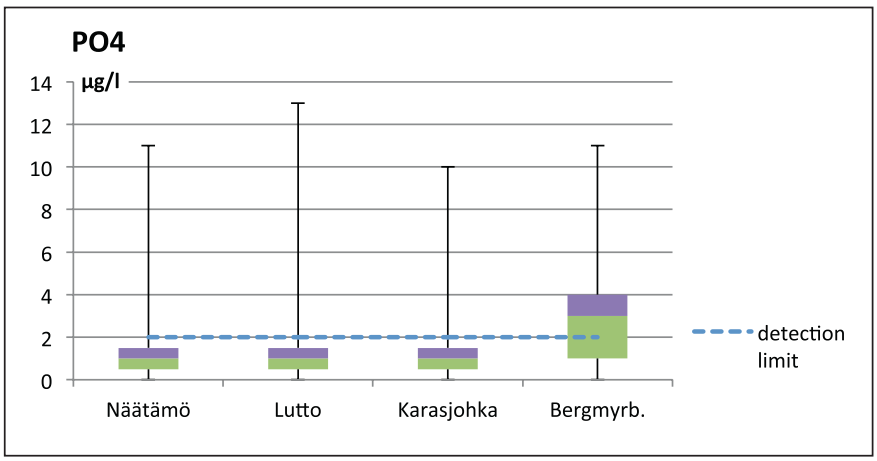


Figure 12. Median phosphate fluctuations in monitored freshwater pearl mussel rivers.

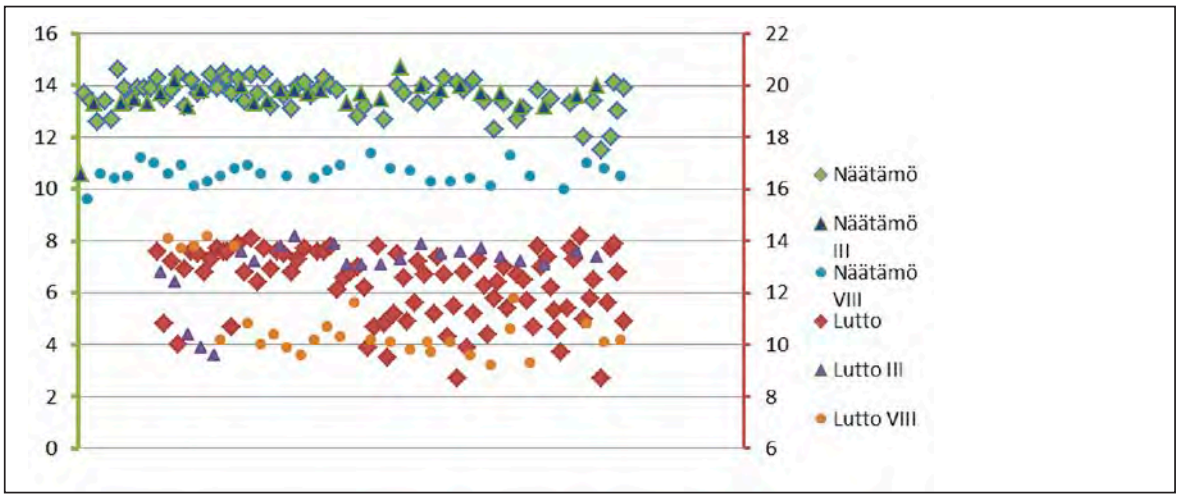


Figure 13. Oxygen (mg/l) in River Näätämö during the sampling period 1980–present and River Lutto during the sampling period 1992–present. March and August presented separately.

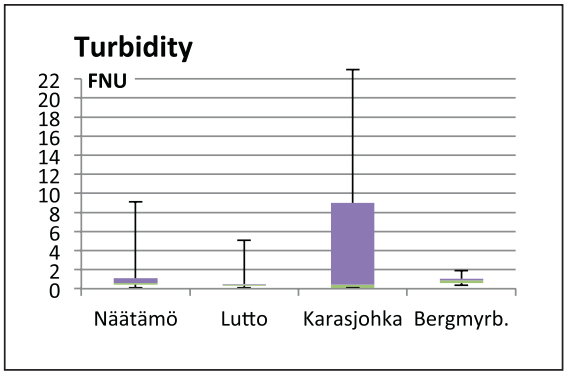


Figure 14a. Turbidity, median and fluctuations in freshwater pearl mussel rivers monitored.

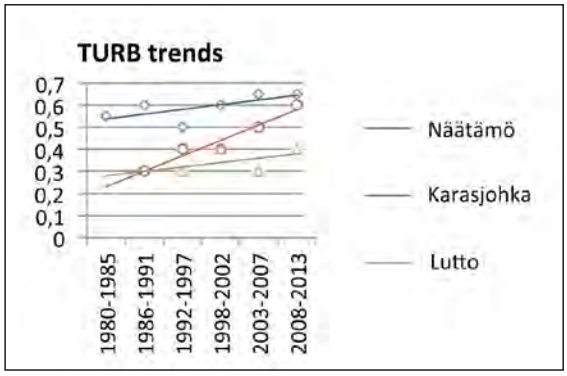


Figure 14b. Turbidity trends in (Y-axis is given in FNU) rivers Näätämö, Lutto and Karasjohka.

from the Lutto appear to be more scattered, since the samples have been taken at different times of year.

Turbidity is normally low, the median not reaching the suggested upper 1 FNU limit for freshwater pearl mussels. Most peaks occur in the Karasjohka and least in Bergmyrbäcken (Fig. 14a). Alarmingly, the trend over the last 20 years is rising in the Karasjohka, Lutto and Näätämö, most clearly in the Karasjohka (Fig. 14b). Turbidity in Bergmyrbäcken is measured only in 2010–2013, and there median turbidity has declined from 1.05 to 0.89 FNU during this time.

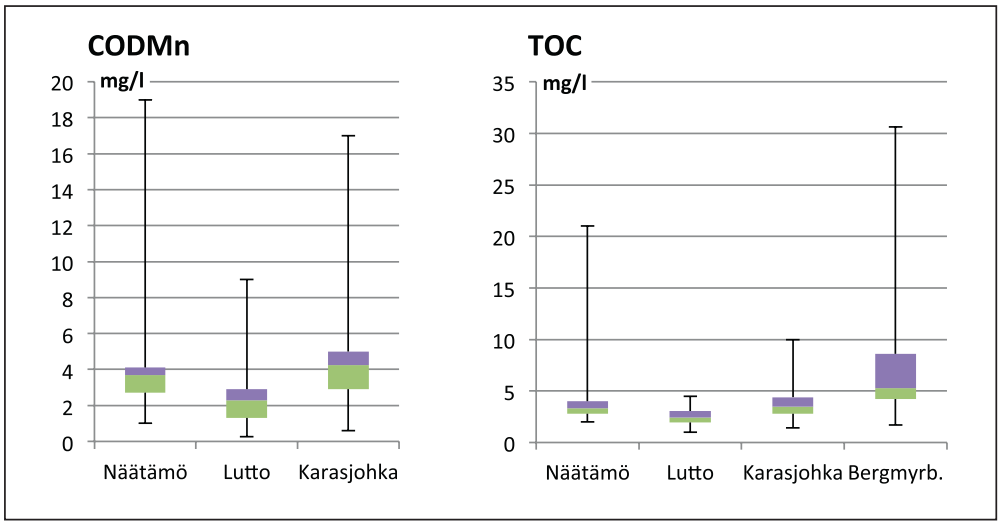


Figure 15. Median chemical oxygen demand (CODMn) and total organic carbon (TOC) in monitored freshwater pearl mussel rivers. These parameters correlate normally quite well, but the TOC measuring period in the Lutto has been 2009–2011 only and CodMn has not been measured in Bergmyrbäcken at all.

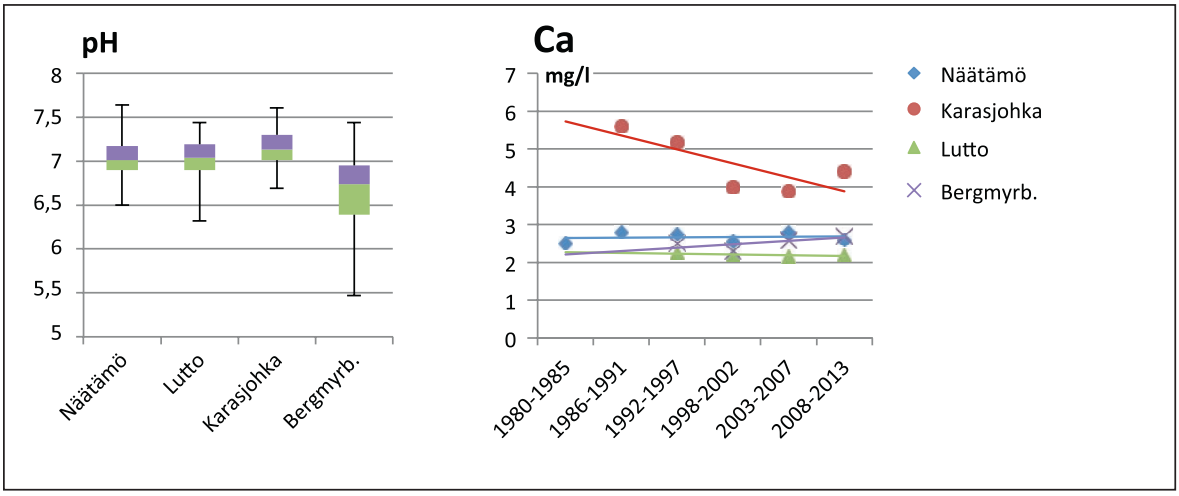


Figure 16. Median pH and calcium (Ca) trends in the rivers compared.

Bioavailability of contaminants depends, among other things, also on the amount of organic carbon in the water column (Thorsen *et al.* 2007). Dissolved organic carbon is not usually measured in Finland, but total organic carbon TOC and chemical oxygen demand CodMn have the lowest levels in the Lutto and highest levels in Bergmyrbäcken (Fig. 15), but a rising trend is observed only in the Karasjohka.

Conductivity is highest in the Karasjohka (median 4.5 mS/m) and lowest in Bergmyrbäcken (median 2.8 mS/m, min. 0.88 mS/m), being quite stable in rivers compared except in the Karasjohka, where the conductivity trend is rising. The median Ca level fluctuates from 2.2 mg/l in the Lutto to 4.65 mg/l in the Karasjohka, but in Bergmyrbäcken and the Lutto also a minimum of 0.6 mg/l is measured. Ca and K levels are stable and similar in the Lutto, Näätämö and Bergmyrbäcken, but a higher level and declining trend in calcium and potassium content and pH is observed in the Karasjohka. However, the low modern average in the Karasjohka is higher than in the other rivers. In Bergmyrbäcken low Ca values are connected with acidic events – even though median pH is close to neutral in all the rivers compared (Fig. 16), reaching its highest values during the summer in the Näätämö and Karasjohka (max 7.6 and 7.4, respectively), and being lowest in Bergmyrbäcken (min. 5.47) during flood events. Still, pH, Ca and K have a slightly rising trend in Bergmyrbäcken.

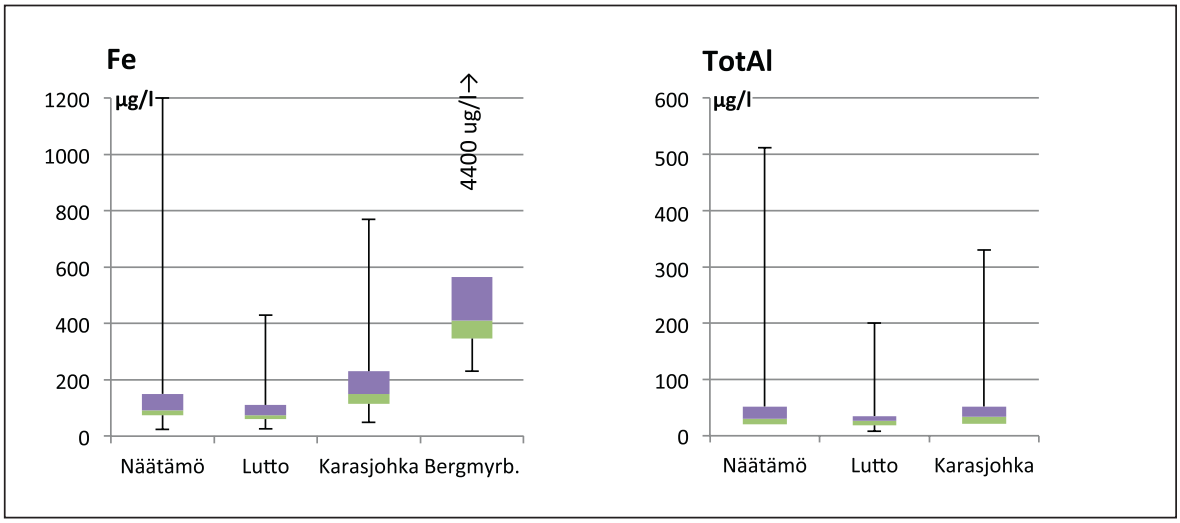


Figure 17. Median iron and total aluminium content in rivers compared.

Bergmyrbäcken also has the naturally highest iron concentration (median 400.5 mg/l with peaks up to 4,400 mg/l), which with low pH can be potentially harmful to freshwater pearl mussels. The Fe content trend in Bergmyrbäcken and Lutto is, however, declining, while in the Näätämö and Karasjohka it is rising. High iron and totAl peaks are also observed in the Näätämö during flood events (Fig. 17). Even though pH in the Näätämö main channel is quite stable, part of the catchment is situated in an acid-sensitive rock area, so acidic events there can be responsible for metal leaching and freshwater pearl mussel population decline in the Näätämö catchment.

Highest SO_4 peaks, up to 100 mg/l is observed in the Karasjohka, while in the Lutto sulphur peaks do not exceed 5 mg/l.

Among the freshwater pearl mussel rivers, where regular monitoring is going on in the target area, Lutto River appears to have the best water quality. So it seems that probably lack of host fish is the main reason for the absence of recruitment in River Lutto.

3.1.4 Seasonal trends in Rivers Karasjohka and Bergmyrbäcken

Month-by-month comparison of the Karasjohka and Bergmyrbäcken (Fig. 18) showed that, even though NO_3 varies similarly in both rivers, Ntot and NH_4N content have their maximum during wintertime in the Karasjohka (as also probably in the Lutto and Näätämö), but during summertime in Bergmyrbäcken. This illustrates that every river is different; which makes understanding from sparse water quality samples even more difficult, and regular water sampling for freshwater pearl mussel monitoring even more important.

3.1.5 Spring series in Rivers Lutto and Kulasjoki

During flood, the content of several hydrochemical components increases in the water column. Peaks during snow-melt, when long transported contaminants, gathered in snow during winter, are melting into the river, are especially dangerous to sensitive biota such as freshwater pearl mussel juveniles. The combined effect with increased content of suspended solids and turbidity, causes stress and maybe also makes juveniles unable to close their shells for long enough to survive through disadvantageous water quality peaks can be the reasons for a lack of recruitment. Monitoring such events is difficult and needs everyday sampling over a long period, so very few data are available about processes and their durations during snow melt, especially from small rivers. Then the water is less mixed, so peaks can be sharper, longer and more dangerous to the freshwater pearl mussel. But if the sub-catchment is in a good condition, more mixed water in the main channel can have worse characteristics due to a mixing of water from some smaller tributaries with unsuitable water quality for freshwater pearl mussels. We still found data from the 1980, when everyday-

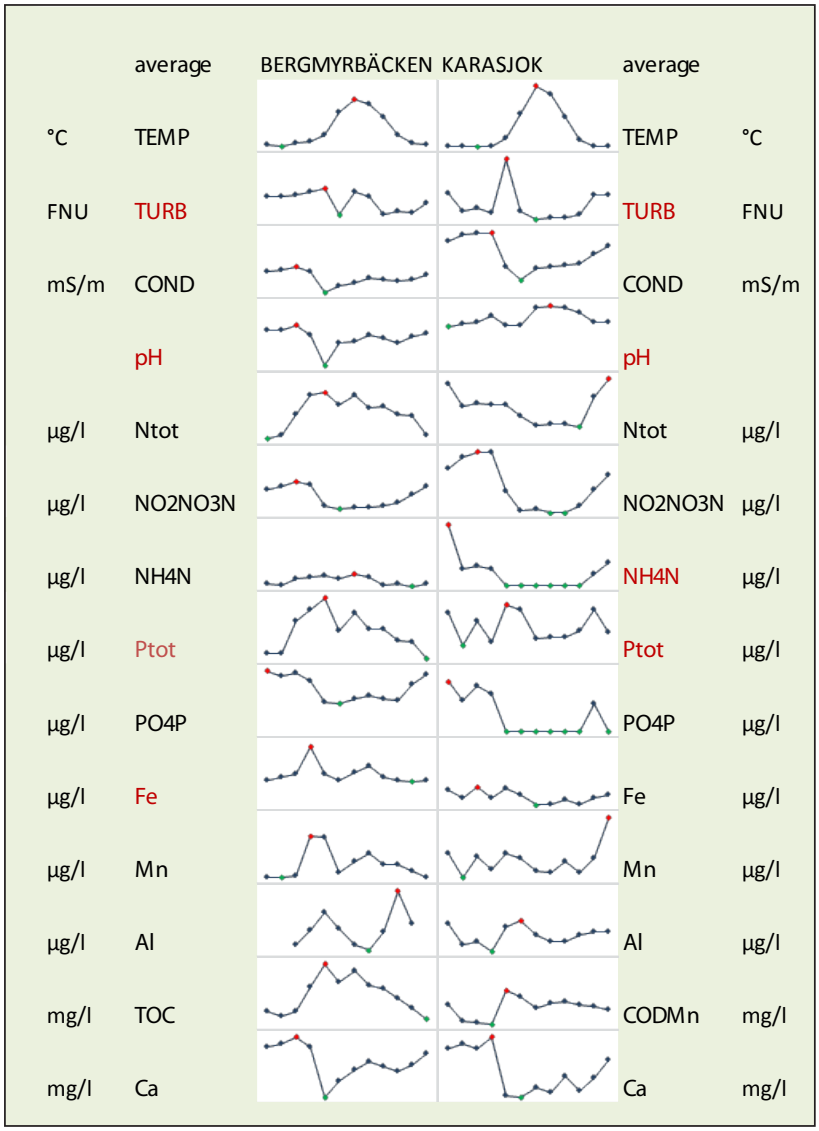


Figure 18. Hydrochemical month by month comparison of the Karasjohka and Bergmyrbäcken. Karasjohka is sampled 1989–1993 2–4 samples per year (IV, VII–IX) and then in 1994–2013 monthly; Bergmyrbäcken is 1995–2004 not sampled in midwinter Dec–Feb., but from 2005–2012 it is sampled monthly.

sampling during spring flood was made in the Näätämö, Lutto and Kulasjoki a (larger side-river of the River Lutto catchment). Even in samples from the River Näätämö main channel, pH was normal, while other parameters indicated acid peaks in the catchment area and tributaries, which might be the reason why we have found freshwater pearl mussels only from the main stream in the Näätämö catchment. Unfortunately, there were no spring series collected from the Näätämö tributaries. The situation was the opposite in Rivers Lutto and Kulasjoki, where water quality was better in the tributary than in the main channel, even though more fluctuations occurred in the tributary (Fig. 19). More frequent and sharper peaks are seen in the Kulasjoki than in the Lutto. Unfortunately, the Kulasjoki is already a big river itself too, so several sequential peaks refer to its smaller tributaries.

3.2 Comparison between rivers

Hydrochemical data about project freshwater pearl mussel rivers in the target area are sparse or missing. For most of the rivers there were only a few samples or/and they were older than 20 years, so they were not really usable as background information. In order to exclude data that was too random or too old, only rivers, where five or more samples from the last 20 years existed, were used for comparison. Hydrochemical information obtained in this way from the Finnish Environmental Database Hertta and from Norwegian authorities and institutions and the respective freshwater pearl mussel population state is presented in Table 2. Probably hydrogeochemically, the pH is naturally high in Skjellbekken, and there are some higher ion levels in Norwegian rivers compared to Finnish ones, as

LUTTO spring serie (border)						KULASJOKI (sub-catchment of LUTTO) spring serie						
1989						1989						
SAMPLING PERIOD		AVERAGE	MEDIAN	MAX	MIN	SAMPLING PERIOD		AVERAGE	MEDIAN	MAX	MIN	mes.unit
28.04.-20.6., n=36						2.05.-20.06 n=36						
TEMP		7,61	8,2	14,9	2,2	TEMP		7,34	7,30	14,80	2,30	°C
TURB		0,52	0,5	1,1	0,2	TURB		0,24	0,20	0,80	0,10	FNU
SS		1,35	1,2	5,9	0,4	SS		0,58	0,50	2,10	0,30	mg/l
COND		2,06	1,965	4,3	1,7	COND		1,81	1,75	2,30	1,50	mS/m
ALK		0,09	0,09	0,127	0,071	ALK		0,08	0,08	0,12	0,06	mmol/l
pH		6,80	6,8	7,1	6,55	pH		6,88	6,88	7,14	6,60	
CNR		20,83	20	35	10	CNR		11,67	10,00	20,00	5,00	mg Pt/l
NO23N		4,36	3	22	0	NO23N		6,36	4,50	24,00	0,00	µg/l
NH4N		2,75	2	9	1	NH4N		2,00	2,00	6,00	1,00	µg/l
Ptot		4,39	4	8	2	Ptot		2,69	2,00	7,00	1,00	µg/l
PO4		0,73	0,7	1,5	0	PO4		0,58	0,55	1,00	0,00	µg/l
Fe		99,47	87	298	54,9	Fe		30,65	29,00	78,60	16,00	µg/l
SO4		2,15	2,1	2,5	1,9	SO4		2,15	2,10	2,50	1,80	mg/l
CODMn		3,48	3,6	4,9	1,8	CODMn		1,84	1,75	3,40	0,90	mg/l

Figure 19. Spring series from Rivers Lutto and Kulasjoki (from the Hertta-database). Low or high values potentially causing stress for freshwater pearl mussel are highlighted in red.

Table 2. Average water quality parameters of some project freshwater pearl mussel rivers (Hertta, Norwegian institute for water research, our data) by population state indicated by colour over the name of the river (population state colour code is given below the table). Colour gradient values of each parameter; white -> yellow indicates low -> high.

Rivers by pop.state nr. of samples		Saukko-oja n=8	Skjellbekken n=17	Spurvbekken n=11	Karpelva n=10	Koutusjoki n=8	Siikajoki n=95	Suomujoki n=5	Livojoki n=22
TURB	FNU	1.30	0.68	0.34	0.58	2.00	0.52	0.23	2.38
COND	mS/m	2.31	9.59	37.83	4.15	2.24	1.64	3.04	3.58
pH		7.04	7.67	7.19	6.87	6.62	6.65	6.97	6.75
CNR	mg Pt/l	48.13	13.47	13.00	30.26	88.13	39.47	7.63	94.05
Ntot	µg/l	217.50	262.00	141.00	139.00	272.50	173.89	53.40	296.09
NO2NO3	µg/l	4.63	22.24	11.82	18.00	9.75	16.06	9.80	28.20
NH4	µg/l	5.13	7.00	3.00	1.00		6.04	2.40	6.05
Ptot	µg/l	14.00	2.32	2.00	3.25	3.69	5.71	3.00	20.45
PO4	µg/l	3.88	1.75	1.00	2.03	2.63	1.26	2.20	10.18
Rauta	µg/l	173.75	29.66	10.10	88.25	1,148.75	200.34	23.75	1,087.39
SO4	mg/l	1.10	6.02	4.31	5.08	1.73	1.30	2.90	1.64
K	mg/l	0.40	0.82	0.53	0.42		0.31	0.38	0.80
Ca	mg/l	2.10	15.39	4.41	2.62		1.21	2.08	4.05
CODMn	mg/l	6.40	3.77	3.35	5.59	12.01	6.03	1.18	11.44
Mg	mg/l	0.50	1.08	0.65	1.00		0.45	0.95	1.35
Na	mg/l	1.70	1.96	1.52	3.22		1.05	1.46	1.89

Population state codes

- viable
- viable/non-viable
- non-viable/viable
- stat.non-viable, but < 20mm freshwater pearl mussel found (at least one)
- stat.non-viable, but < 40mm freshwater pearl mussel found (at least one)
- stat.non-viable, but < 50mm freshwater pearl mussel found (at least one)
- dying out

Table 3. Results of water analysis autumn 2013 by population state code (population state colour code is given in Table 2). Colour gradient values of each parameter; white -> yellow shows low -> high.

Results of water analysis 2013

Mm pop.state	Hanhi-oja	Uusi-joki	Kopsus-joki	Hirvas-joki	Kiertama oja	Kiertama oja upper	Torko-joki	Juo-joki	Kuutus-oja	Suomu-joki	Lutto 2 lower	Lutto upper	Kivijoki
Date	06.10.13	19.10.13	20.10.13	19.10.13	12.10.13	12.10.13	19.10.13	29.09.13	19.10.13	19.10.13	17.10.13	19.10.13	06.10.13
Cond25(mS/m)	3.2	3	5.1	4.2	4.2	3.3	4.7	2.7	3.6	3.3	3.8	3.9	4.8
Alk(µeq /l)	200	195	392	288	262	239	322	148	236	199	249	252	364
pH	7.1	7	7.12	7.18	6.94	7.18	7.13	6.8	7.18	7.11	7.11	7.18	7.36
Color o	27	11	16	10	7	10	21	61	8	4	8	7	9
totN(µgN/l)	158	134	72	145	464	88	215	276	131	37	99	101	94
NO3(µgN/l)	10	11	0	9	2	1	16	2	9	5	4	4	3
NH4(µgN/l)	4	7	1	21	115	8	21	18	2	2	7	7	4
totPnfilt.(µg/l)	9	5	10	9	15	7	13	19	7	9	5	7	7
totPfiltr.(µg/l)	9	4	8	5	13	7	13	10	4	8	5	7	5
PO4P(µg/l)	7	4	6	6	10	6	11	5	5	7	5	7	5
totFe(µg/l)	77	53	116	102	33	48	73	750	40	18	80	44	48
SO4(µg/l)	3.24	2.66	4.37	3.99	2.62	2.8	4.24	1.75	3.53	3.88	3.82	4.48	3.08
totAl(µg/l)	30	19	14.6	36	9	14	30	90	9.5	6	20	20	17.5
K (mg/l)	0.55	0.5	0.75	0.5	0.95	0.4	0.75	0.6	0.5	0.5	0.6	0.5	0.7
Ca(mg/l)	2.78	2.44	4.48	3.57	2.72	2.76	4.27	2.36	2.81	2.63	3.11	2.98	4.64
COD Mn(mg/l)	5.66	3.94	3.33	3.3	2	2.64	6.16	13.17	3.48	1.88	2.68	3.54	2.32
Cl(mg/l)	1.09	0.98	0.84	1.11	2.59	1.09	1.09	0.92	0.84	0.87	0.99	1	1.31
Mg(mg/l)	1.24	1.09	2.13	1.51	1.33	1.29	1.69	0.91	1.53	1	1.36	1.53	1.76
Na(mg/l)	1.57	1.61	2.38	2.22	3.2	1.65	2.38	1.43	1.61	1.65	2	1.78	1.78
TOC(mg C/l)	5.9	4.6	4.1	4.1	3.1	3.6	6.3	11.6	4.2	3	3.6	4.3	3.3
Si (mg/l)	3.25	2.35	6.17	3.82	3.72	3.69	3.74	2.57	3.18	3.55	3.35	3.75	3.83
totCu (µg/l)	0.3	0.2	<0.2	0.2	0.8	<0.2	0.2	0.3	0.2	<0.2	0.2	0.2	<0.2
totCd(µg/l)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
totCr(µg/l)	0.3	0.2	0.3	0.3	0.6	0.7	0.2	0.4	0.4	0.4	0.3	0.3	0.2

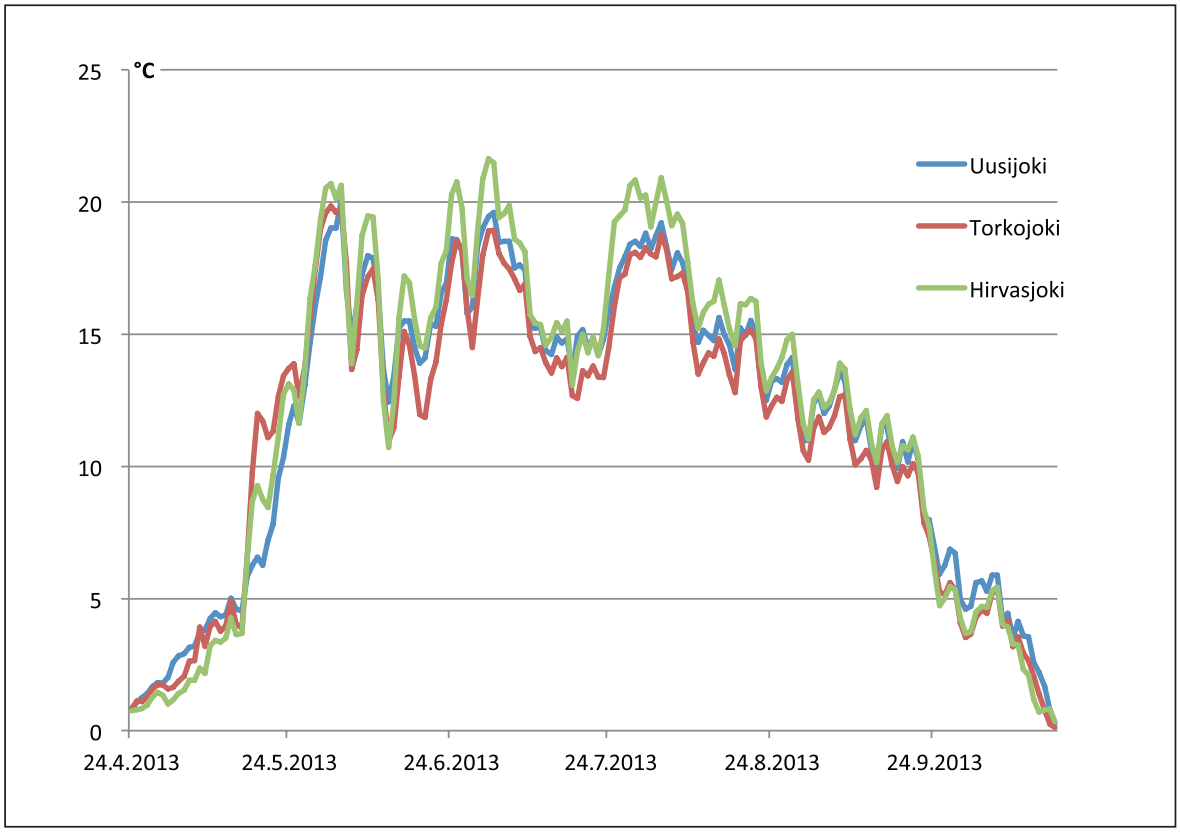


Figure 20. The temperature curves through the ice-free season of the three Finnish freshwater mussel rivers, Uusijoki, Torkojoki and Hirvasjoki, are shown.

they are influenced by greenstone bedrock belt (see Fig. 1) but turbidity, nutrients and organic matter is lowest in River Suomujoki, a tributary of the Lutto. This suggests, as we saw in the case of River Lutto (see Regular monitoring above), that lack of recruitment could be mainly caused by the lack of host fish, as salmon ascending to these rivers are blocked.

3.3 Analyses conducted in this study

3.3.1 Water quality samples

The water quality of samples, taken in the autumn of 2013, was good, with slightly elevated phosphate in the Torkojoki (11 µg/l) and in the lower course of the Kiertämäoja (10 µg/l) (Table 3). Ptot was also elevated in both rivers (13 and 15 µg/l, respectively) and in addition in the Juojoki (19 µg/l). Juojoki also had high iron content (750 µg/l). The iron capacity of binding orthophosphate can be the reason why the phosphate level was low, even though Ptot was elevated. The highest Ntot was found in the lower course of the Kiertämäoja (464 µg/l) and lowest in the Suomujoki (37 µg/l). Total ammoniacal nitrogen (TAN) was also highest in the Kiertämäoja (115 µg/l). Hirvasjoki, Torkojoki and Juojoki showed also a little higher TAN and total nitrogen content than other rivers. Our samples give a first hint of the hydrochemistry and water quality for several rivers. Comparing those samples with freshwater pearl mussel status, one can see that the worst water quality is in rivers where the freshwater pearl mussel population status is non-viable, but some small freshwater pearl mussels are found, so probably these populations are declining due to current water quality status. In any case, not much can be said about water quality in a river from a single water sample, especially because a single missed short-term pollutant peak can have long-term consequences in the river ecosystem. Regular monitoring of freshwater pearl mussel rivers is necessary for future monitoring and maintaining freshwater pearl mussel populations, as well as avoiding activities that results in degrading water quality in freshwater pearl mussel rivers. Every river is a unique complexity of factors, that can have amazing values and still work if everything is in balance. This equation is somewhat different for every river and the freshwater pearl mussel is among first ones to have problems with recruitment if the parameters are changed.

3.3.2 Series from temperature loggers

In Figure 20 the temperature curves through the ice-free season of year of the three Finnish freshwater mussel rivers Uusijoki, Torkojoki and Hirvasjoki are shown. River Uusijoki appears to have 2072 day-degrees, Torkojoki 1999 day-degrees and Hirvasjoki 2143 day-degrees. River Hirvasjoki has the higher temperature, being higher than 20°C on several days. The Torkojoki appears to have the best recruitment and strongest population of freshwater pearl mussels.

Further, data from these loggers also provides information on various chemical processes. The temperatures influence the oxygen saturation in the water. Colder water contains the highest oxygen concentrations and then the concentration will decline with increasing temperature. Above 20°C, the oxygen concentration in the sediment tends to become lower than is required by young mussels.

3.3.3 Heavy metals in the water

From the results presented in Table 4 and Figs 21, 22 and 23, it is seen that the highest values for Al, Cd, Cu and Ni and Zn were found in River Karpelva. All the rivers in Finland were very low in Cu and Ni. In River Lutto a high variation (between spring and summer) was found in for Co, Pb, Sr and Zn. Most rivers have elevated levels of metals in the spring period, in many cases much higher values than in the summer. However, Strontium (Sr) mainly has some higher levels in the summer period in most of the rivers. Strontium exhibited a great variation in Rivers Lutto, Hirvasjoki and Uusijoki.

In River Lutto, the metals Co, Pb, Sr and Zn appear to have great variation. Rivers in Finland exhibit the lowest levels of copper (Cu) compared to Norwegian rivers and the Swedish river. The Norwegian rivers all had the highest level of nickel (Ni) – except Neiden/ Näätämö. Manganese (Mn) is highest in Skjellbekken, followed by the Utsjoki, Uusijoki and Karpelva. Lead (Pb) had the highest variation in the Lutto,

Table 4. The heavy metal concentrations calculated for running river water by the DGT samples.

Time	River	Al µg/l	Cd µg/l	Co µg/l	Cr µg/l	Cu µg/l	Fe µg/l	Mn µg/l	Ni µg/l	Pb µg/l	Sr µg/l	Zn µg/l
Spring	Karpelva lower	29	0.073	0.23	0.08	5.9	48	4.6	76	0.013	0.61	12
Summer	Karpelva lower	1.6	0.0045	0.0049	<0.01	0.68	2	0.49	4.5	0.0016	0.17	0.61
Spring	Karpelva upper	11	0.049	0.16	0.02	5.4	20	2.1	54	0.0031	0.63	8.5
Summer	Karpelva upper	2.0	0.0048	0.0056	<0.01	0.92	2	0.78	5.1	0.00037	0.21	0.59
Spring	Neiden	0.87	0.00050	0.0015	<0.01	0.041	<1	0.44	0.069	0.00020	0.23	0.10
Summer	Neiden	0.71	0.00047	0.00040	<0.01	0.054	<1	0.17	0.069	0.00049	0.34	0.18
Spring	Skjellbekken	3.9	0.0033	0.013	0.03	0.31	9	6.4	0.42	0.014	0.11	0.39
Summer	Skjellbekken	0.65	0.0015	0.0039	0.01	0.23	2	3.9	0.49	0.00069	0.19	0.51
Spring	Spurvbekken	0.41	0.0029	0.0020	0.01	0.17	<1	0.32	0.29	0.00077	0.12	0.34
Summer	Spurvbekken	1.8	0.0014	0.00074	<0.01	0.14	<1	0.27	0.25	0.00047	0.19	0.81
Spring	Ørnebekken	0.70	0.0031	0.0022	<0.01	0.50	<1	0.59	1.5	0.0014	0.11	0.24
Spring	Uusijoki	7.9	0.00053	0.028	0.04	0.14	27	3.9	0.052	0.0066	0.026	0.36
Summer	Uusijoki	1.4	0.0011	0.0082	<0.01	0.020	2	0.77	0.070	0.00041	0.19	0.28
Spring	Luttojoki	0.55	0.0020	0.026	0.03	0.056	4	1.6	0.22	0.022	0.63	1.4
Summer	Luttojoki	0.70	0.00092	0.012	<0.01	0.028	<1	1.1	0.14	0.00044	0.19	0.17
Spring	Hirvaskoki	1.5	0.0015	0.016	0.03	0.028	4	0.51	0.10	0.00097	0.15	0.64
Summer	Hirvaskoki	2.1	0.0010	0.00098	<0.01	0.026	<1	0.21	0.050	0.0011	0.37	0.27
Spring	Torkojoki	4.0	0.0014	0.0091	0.03	0.033	7	1.1	0.12	0.0015	0.26	0.76
Summer	Torkojoki	2.1	0.00052	0.0020	<0.01	0.017	1	0.27	0.067	0.00029	0.36	0.13
Spring	Utsjokha	4.1	0.00083	0.0090	0.02	0.079	9	3.7	0.062	0.0019	0.92	0.37
Summer	Juojoki	2.5	0.0017	0.017	<0.01	0.034	12	1.7	0.077	0.00045	0.15	0.43

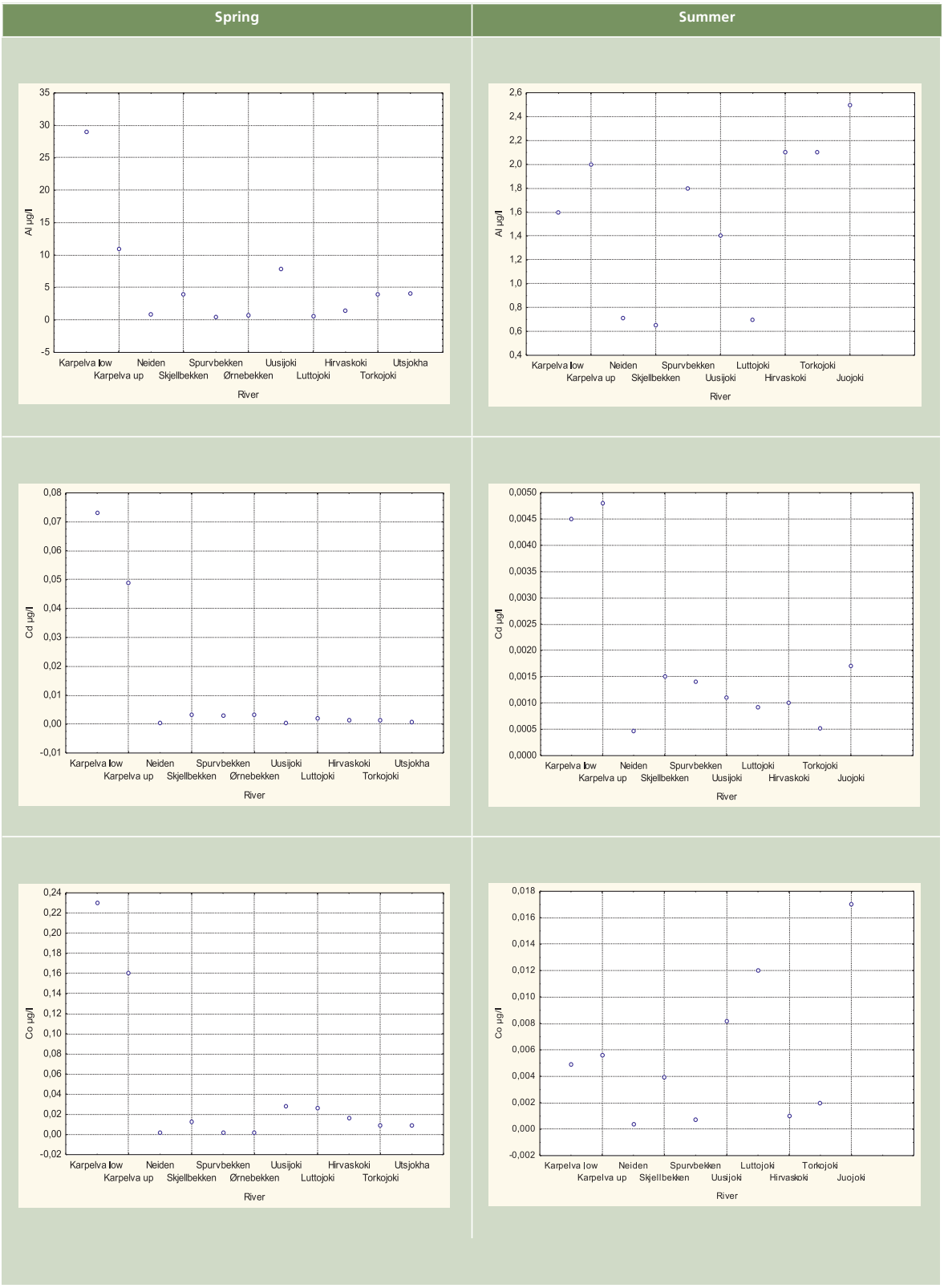


Figure 21. Spring (Left-hand side) and summer (right-hand side) DGT values of aluminium (Al), cadmium (Cd) and cobalt (Co) from the target rivers. Note that results from some rivers/seasons are missing: Ørnebekken (summer), Juojoki and Utsjoki (spring), and Fe and Cr due to many values below the detection limits. NB: The Y-axis shows various scales in spring and summer samples.

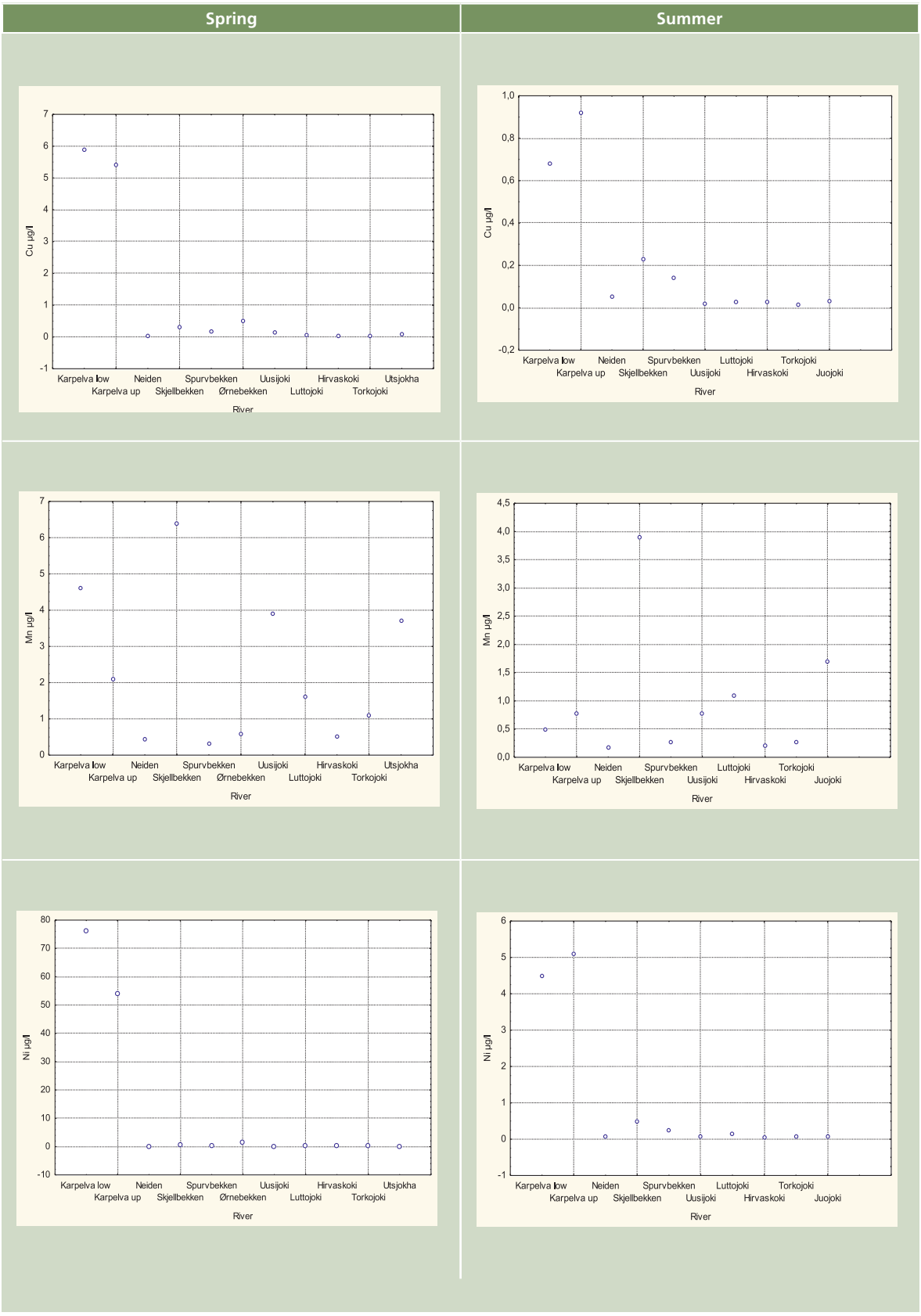


Figure 22. Spring (Left-hand side) and summer (right-hand side) DGT values of copper (Cu), Manganese (Mn) and nickel (Ni) from the target rivers. NB: The Y-axis shows various scales in spring and summer samples.

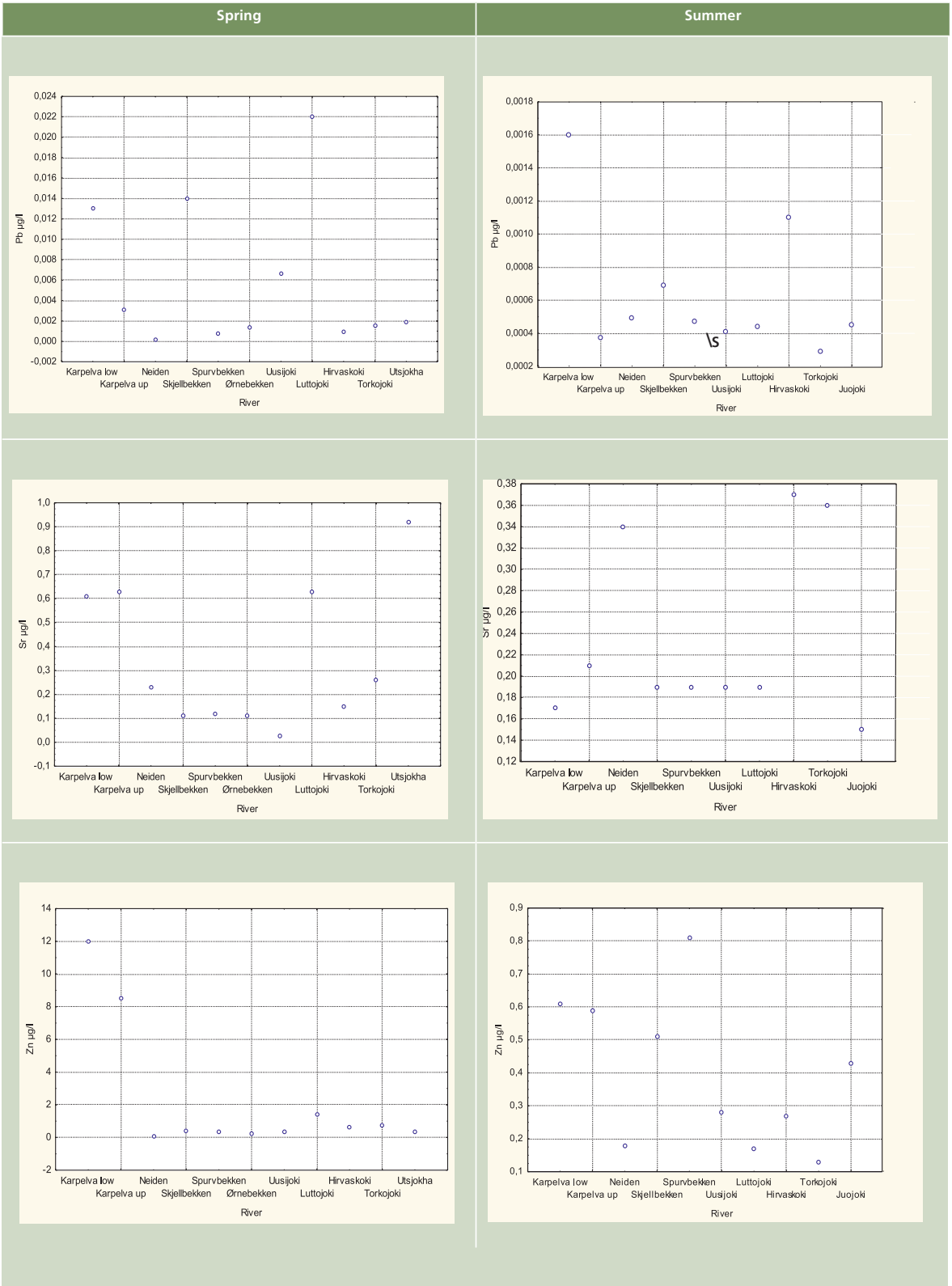


Figure 23. Spring (Left-hand side) and summer (right-hand side) DGT values of lead (Pb), Strontium (Sr) and zinc (Zn) from the target rivers. Note that results from some rivers/seasons are missing: Ørnebekken (summer), Juojoki and Utsjoki (spring), and Fe and Cr due to many values below detection limits. NB: The Y-axis show various scales in spring and summer samples.

Figure 24. One site where young freshwater pearl mussels are present in River Skjellbekken. The mussels in the black net were collected from the background. The sediment sample was taken from the same place. Photo Paul Aspholm.

Skjellbekken and Uusijoki. Chromium (Cr) and iron (Fe) appeared with low levels and some data below the detection limit.

3.3.4 Heavy metals in the sediment

The results of the analysis do not show any clear systematic pattern in the concentrations of heavy metals in the sediment from the various rivers and when comparing sites with adults and young mussels. However, at sites with young mussels (Fig. 24) some tendencies to slight reductions can be seen in some of the rivers, while there is an increase in some other rivers. Interestingly, the concentration of iron (Fe) in the sediments at sites with young individuals appears to be around 11 mg/g. Sediments at mussel sites in the Neiden have the highest iron concentration compared to the other rivers. In River Neiden nickel (Ni), zinc (Zn) and arsenic (As) are low, while calcium (Ca) and magnesium (Mg) are high compared to the rivers where there are young recruiting mussels. The results are shown in Table 5.

Table 5. Sediment samples taken in the layer of 0–5 cm depth at sites in the vicinity of young freshwater pearl mussels (less than 20 mm) presented in the column coloured green. The samples in blue-coloured cells are from the same river section (20–50 m apart) where adult mussels only were detected. The rivers were the Finnish part of River Näätämö/Neiden where mussels occurred. Spurvbekken and Skjellbekken are Norwegian rivers and Krakojoki is a tributary of Skjellbekken. These results do not explain the relation between the heavy metal concentrations in the sediments and the occurrence of young mussels, and nor do they explain the reason for the lack of recruitment in the River Neiden. The iron concentration in sediments in the Neiden is high, though this element could possibly be regarded as important for its buffering capacity in relation to the other heavy metals. Together with dissolved organic carbon (DOC), the iron complexes may reduce the bioavailability of the more toxic metals.

Sediment samples							
	Näätämö Neiden	Spurvbekken1	Spurvbekken2	Skjellbekken 1	Skjellbekken 2	Krakojoki 1	Krakojoki 2
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Cu Copper	12.8	23.1	17.9	7.69	10.3	10.3	7.69
Ni Nickel	13.7	46.6	24.7	13.7	13.7	19.2	19.2
Zn Zinc	29.4	141	92.3	21	21	23.8	26.6
Mn Manganese	213	213	925	238	225	163	225
Fe Iron	16,271	14,576	10,690	13,793	10,690	9,655	1,1379
As Arsen	0.33	2.65	2.66	1.18	1.08	1.32	1.18
Hg Mercury	0.004	0.006	0.008	0	0	0.006	0
P Phosphates	160	162	168	476	192	230	226
Ca Calcium	5,680	3,360	2,720	4,800	4,880	3,760	3,920
Mg Magnesium	4,211	2,674	2,400	3,580	3,068	3,102	3,409

This implies that the young mussels may tolerate relatively high concentrations of heavy metals in the sediment where they live, and specific processes and factors may occur that neutralize the toxic effect of high metal concentrations. The observed results of the heavy metals in this study indicate tolerant levels, though this may not be the case in other rivers outside the study area.

A major question that remains unanswered is: What will happen during acid events and periods with substantial changes in the chemical properties, e.g. during increased nitrogen availability such as during autumnal die-out of filamentous macro-algae? The microhabitats of young mussels probably have some properties making them especially preferred and advantageous for recruitment.

The annual autumn floods may have similar functions, although the mussels may be more exposed and vulnerable to the effects since their metabolism is higher and they are more actively filtering the water at this time of the year. More knowledge is needed about the function of various processes occurring in the waters, sediments and substrates. What happens, for instance, during flood? The annual spring flood may transport winter-accumulated nutrients and components through the river system, resulting in acidic, nitrogen or fine material peak events. During these events the content of heavy metals may be hazardous. On the other hand, the flood often washes out and renews the sediments and, as well as resulting in higher oxygen.

3.3.5 Heavy metals in freshwater pearl mussel foot

We have in this study chosen the foot as the indicator of soft tissue, since it is the most optimal part of the organism to use while being less potentially influenced by temporary environmental conditions, fluctuations or occasions such as food in the digestive tract, or DOC-bound metals in the mucus of the gill surface, etc.

In our results, the most striking findings of heavy metals in the tissue of the mussels are the high concentrations of nickel (Ni) and chrome (Cr) in the Swedish River Juojoki (Fig. 25). The mussels from the Norwegian Rivers Karpelva (Kelv), Skjellbekken (SkB), Spurvbekken (SpB) Føllelv (Felv) had higher concentrations of copper (Cu), cadmium (Cd), lead (Pb), mercury (Hg) and zinc (Zn) than those from the River Juojoki. Among the Norwegian rivers, Karpelv had the highest concentrations of copper (Cu), cadmium (Cd) and mercury (Hg) and almost as high nickel (Ni) concentration as the River Juojoki.

3.3.6 Heavy metals in the freshwater pearl mussel shells

The results of the analyses of heavy metals and nutrients were analysed in freshwater pearl mussel shells from 131 individuals from six rivers: the Skjellbekken, Spurvbekken, Føllelva (in Norway), and the Juojoki and Ljusträskbäcken (in Sweden) and one old shell from River Lutto (in Finland).

In Figure 25, the concentrations of calcium (Ca) are given in mg/g for shell and foot. The average Ca concentration in shells of the populations ranged from 354 mg/g to 368 mg/g, where samples from the River Juojoki have the highest Ca concentration. There is a great variation in the results with overlap of outlier and extremes from the different rivers. Interestingly, the single shell from the River Lutto has a relatively high Ca concentration compared to those shells from the other rivers. The concentrations of aluminium (Al), calcium (Ca) and copper (Cu) are given in Figure 25, and mercury (Hg), magnesium (Mg) and nickel (Ni) in Figure 26. River Føllelva mussels reveal high concentrations of aluminium in the foot, but near average concentrations in the shell. Freshwater pearl mussels in Skjellbekken have the highest average and also a large variety of aluminium content in the shell. Spurvbekken mussels are found to have the same average as the value in the old shell from the Lutto.

From a Principal component analysis (PCA) of 18 selected heavy metals and components in freshwater pearl mussel shells (Fig. 27) it is seen that the most important of these metals were Al, V, K, Sn, Mg, Cr (to the left along the PC1-axis) and Cu, Hg, Mo on the other end of this axis (PC2-axis). The rivers appeared along the same PC1-axis in the following order: Juojoki (Jj), Føllelva (Felv), Skjellbekken (SkB), Ljusträsk-

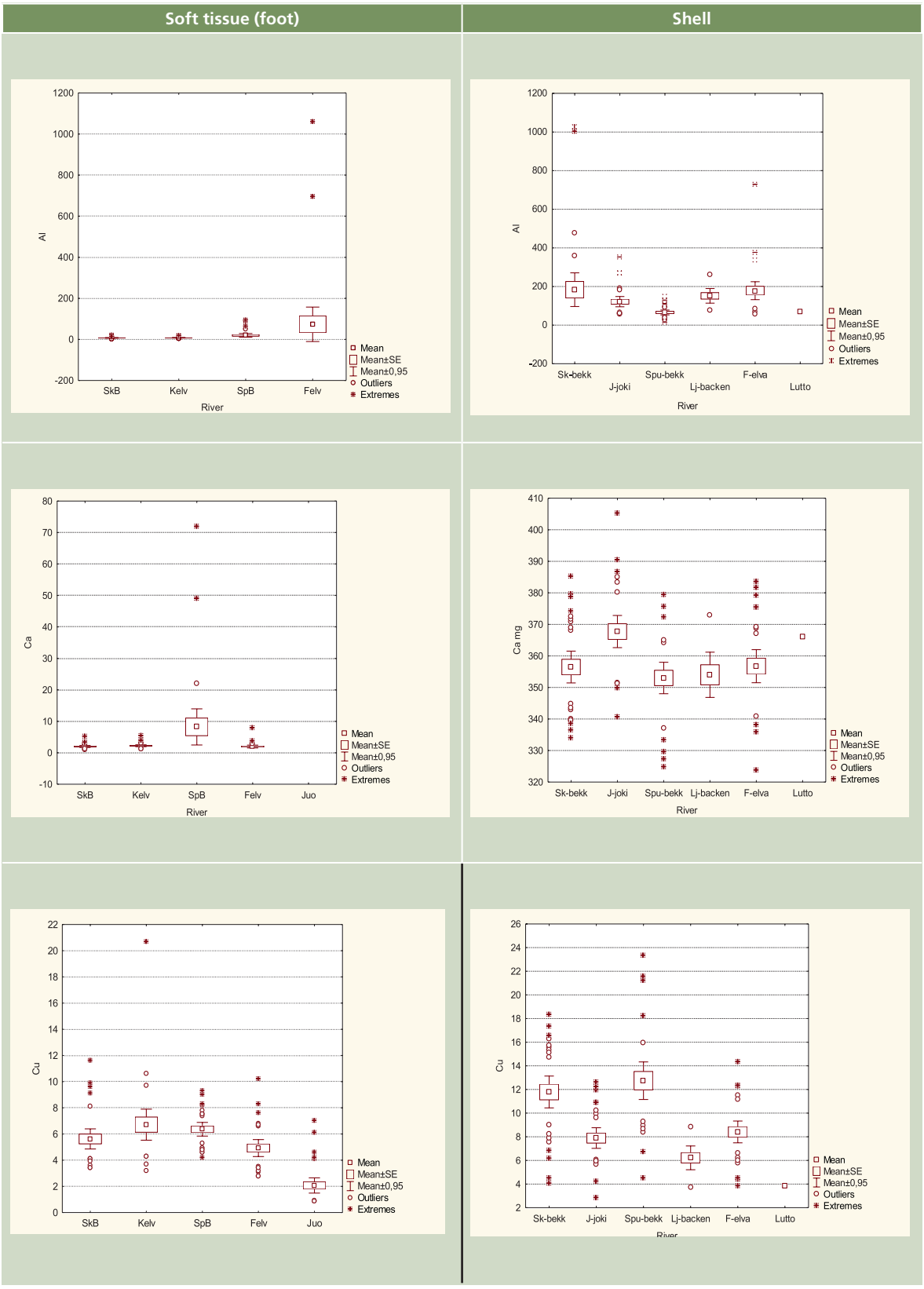


Figure 25. The concentrations of aluminium (Al), calcium (Ca) and copper (Cu) in the foot (left-hand side) and shell (right-hand side) from Skjellbekken (SkB/Sk-bekk) Spurvbekken (SpB/Sp-bekk), Føllelva (Felv/E-elva) and Juojoki (Juo/J-joki). From the Karpelv (Kelv) only results from the foot are shown, while from Ljusträskbäcken (Lj-backen) and the Lutto only results from shell are shown.

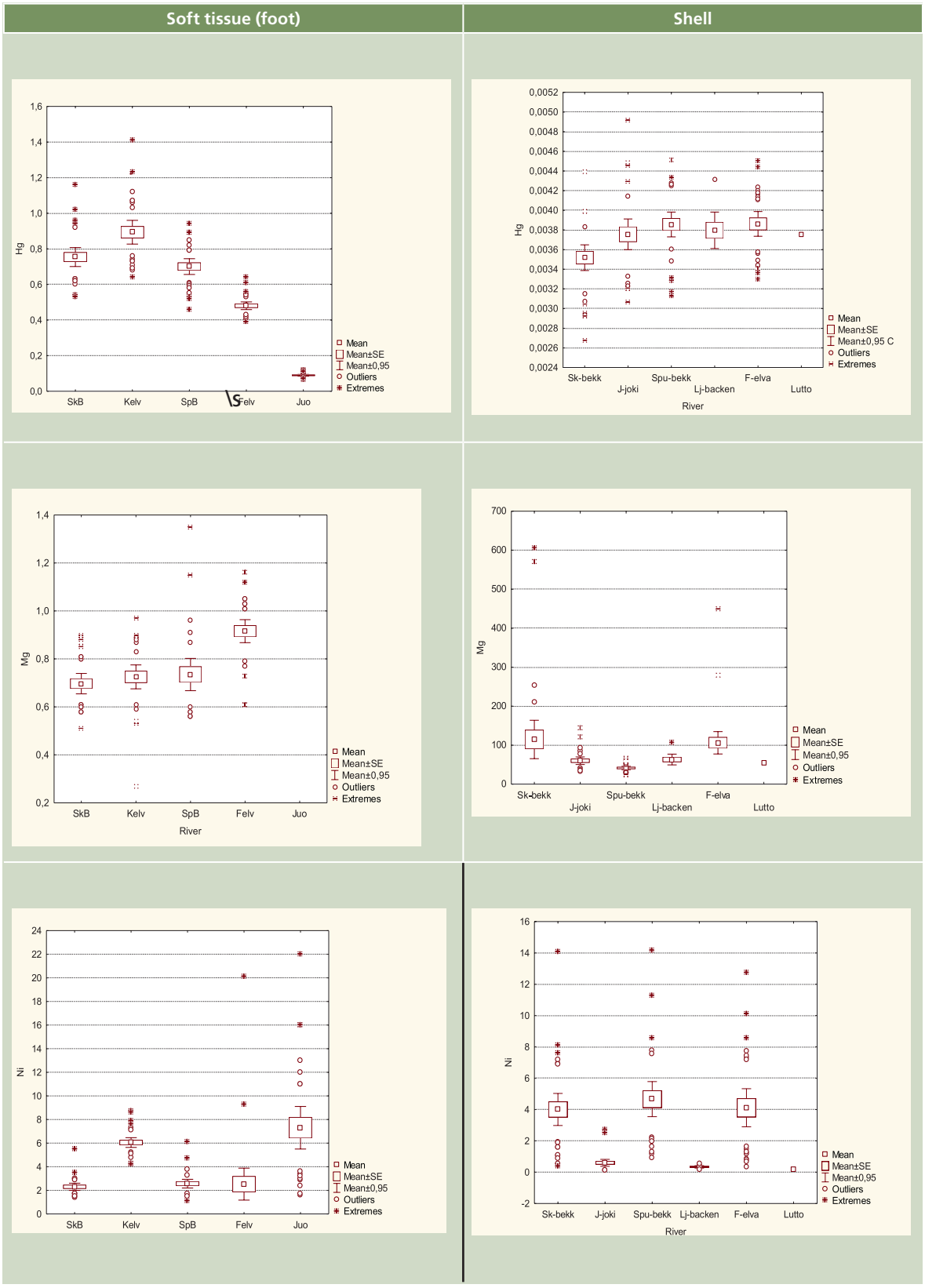


Figure 26. The concentrations of mercury (Hg), magnesium (Mg) and nickel (Ni) in the foot (left-hand side) and shell (right-hand side) from Skjellbekken (SkB/Sk-bekk) Spurvbekken (SpB/Sp-bekk), Føllelva (Felv/E-elva) and Juojoki (Juo/J-joki). From the Karpelv (Kelv) only results from the foot is shown, while from Ljusträskbäcken (Lj-backen) and Lutto only results from the shell are shown. NB: Different scales on the Y-axis from the soft tissue and shell.

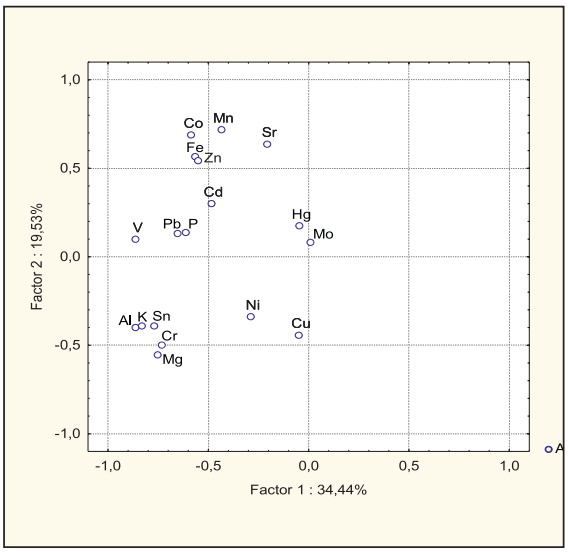


Figure 27: Heavy metals and components in freshwater pearl mussel shells. PC1 factor (35%) in the following order: Al, V, K, Sn, Mg, Cr, Pb, P, Co, Fe, Zn, Cd, Mn, Ni, Sr, Cu, Hg and Mo.

bäcken (LjB), Spurvbekken (SpB) and Lutto (Luo) (Fig. 28).

The most obvious results were that freshwater pearl mussel shells were high in Cu from Skjellbekken and Spurvbekken, and those high in Ni were from Skjellbekken, Spurvbekken and Føllelva, river systems in NE Norway. Also, other heavy metals differed in the shells from different rivers, but to a less obvious extent. The highest Ca concentration was found in freshwater pearl mussel shells from Juojoki in Sweden. Even though there is only one shell in the sample, it is interesting that the old shell from River Lutto is showing low levels of most heavy metals. In most cases it is seen to correspond to the least contaminated individuals in each of the populations. The only exception is the level of mercury (Hg) where the Lutto shell is similar to the mean of most populations, and above the average of Skjellbekken. In addition to mercury, this shell also shows a high content of calcium (Ca).

To summarize: there were no clear correlations between the concentrations in the soft tissue (foot) and the shell of the individual mussels. This is due to high individual variations of metals. The timescale represented in the shell and foot of the mussels is likely to explain this lack of correlation within each mussel. However, the average levels of copper in Skjellbekken and Spurvbekken are high. Among the freshwater pearl mussel populations in the rivers investigated it seems that aluminium (Al) is one of the metals that appears differently in the shells of the various populations. It is striking that there are some individuals that have high levels of some of the metals and that the pattern of contaminants is highly variable. No systematic rank or relation appears between the different contaminants when they occur with high concentration. This may indicate that there are different incidents on how the contamination appeared and developed in each individual.

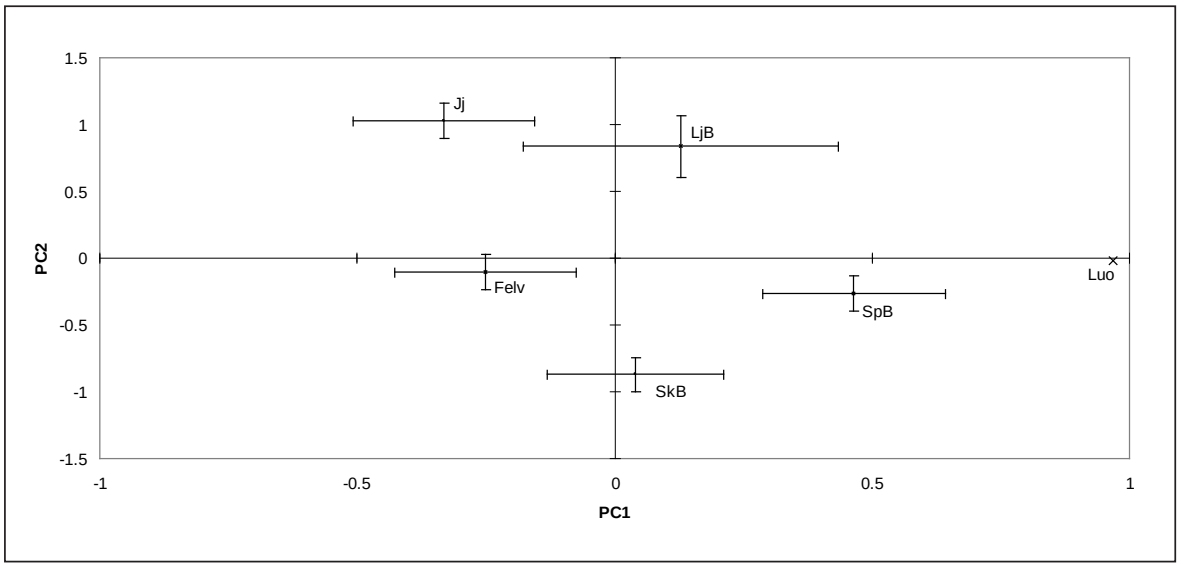


Figure 28. Principal component analysis of the concentration of heavy metals and elements in mussel shells in rivers.

4 Implications and conclusions

The findings through this project indicate a pollution effect in some of the rivers. Natural background levels are highly variable between rivers. Furthermore, even the less human-influenced rivers perform seasonal fluctuations in their parameters. One of the major effects is the increase in observed acidification events. This acidification is not only driven by precipitation; there seems to be an increasing effect possibly of nitrogen components that influence the water quality towards acidification at times. Then consequences are the release and transformation of aluminium to a toxic form of aluminium. Together with the release of organic components from land use in many areas, this results in events of toxic aluminium components bioavailable for uptake in the freshwater pearl mussel. These events with high organic content in the water also influence oxygen availability in the potential young freshwater pearl mussel habitats. Nutrients like nitrogen and phosphate from local land use and long transport, have a negative influence. Also the fish hosting the glochidia (mussel larvae) are influenced by water chemistry as well as fishing and water management influence the densities and abundance of the hosts and the subsequent reproduction possibilities for the freshwater pearl mussels.

The climate prognoses predict changes in precipitation and temperature to increase. One especially important feature is the multiple alternating thawing and freezing during winter and early spring which is expected to be more frequent. This in turn increases the release of nutrients, heavy metals and particles from the land so that they are more bioavailable in the river water. Along with an increase of organic sedimentation, increasing temperature will reduce oxygen concentration in the water.

Our results from investing the data-series of water qualities and the analyses of DGT sediments and contamination in freshwater pearl mussels indicate that there has been some influence from the Russian nickel smelting factory on Rivers Karpelva, Skjellbekken, and Spurvbekken and possibly Føllelva in Norway and River Näätämö in Finland by heavy metals like nickel, copper, cobalt and arsenic. There is a possibility that River Juojoki has been influenced by the Finnish ferrochrome smelter in Tornio. Today the influence of airborne heavy metals is minor in all rivers except River Karpelva. The major effect is the contribution to acidification. Naturally, the bedrock and soil substrate have a buffering capacity that in many ways could deal with the regional industrial emissions. However, there are signs of increasing nitrogen components that are transported from a distance and local sources connected to agriculture and forestry activities. These nitrogen components arise in several different processes such as acidification under various conditions. These acidification events are for instance spring peaks. During these spring peaks, more heavy metals become bioavailable as they are transferred from the land to the river ecosystem by the flooding. The increased agricultural and forestry activity makes for more runoff of nutrients and material into the rivers. Climate is influencing higher turnovers of nutrients and pollutants and their bioavailability. One such phenomenon is that there is possibly more nitrite in the sediments where the young mussels are living. The reduced recruitment in freshwater pearl mussels is in most of our area, as in River Näätämö, possibly a long-term process (several years in a row) of peaks (lasting some few days or even less time) where the pH is low. Then the increased availability of heavy metals possibly combined with increased levels of water quality parameters such as nitrogen and phosphates have contributed to the reduction of the population. During the fieldwork, temperature in sediments was also measured. There is a trend that the spots inhabited by young mussels have a somewhat warmer temperature in the sediment in summer at five and ten centimetres depth compared to spots where there are no young mussels occurring. This may indicate that these are spots where the river water is coming up in the streambed, compared to the colder groundwater. In addition, a movement of water from sediment to river flow might reduce clogging of the sediment surface. It is also noted that the sites of young mussels often have a higher frequency of earthworms (Lumbricidae), which indicates high oxygen content. During the planning of restoration measures, it might then also be important to secure the down flow and up flow of stream water through the riverbed,

and as well to ensure that such conditions are not destroyed during restoration.

Dissolved organic carbon (DOC) is an important factor in the food for the very young freshwater pearl mussels. DOC has also an important function in reducing the bioavailability of some pollutants, though it might make other pollutant more bioavailable. Nevertheless, it is important to carry out the restoration in such a manner that DOC is available at the same level as prior to human interference. DOC might originate from mires and wetland, so it is important to keep these elements in the catchment as a natural function.

5 Recommendations

What to do when restoring: It is indicated that measures must be taken to reduce the nutrient leaching from land to river in order to achieve success in restoring the freshwater pearl mussel population. This can be done by protection and buffer zones along the water bodies and by nutrient catchment ponds to collect the excess nutrients coming from forest and agriculture. This type of measure will reduce the particles released during snow-melt periods, spring and autumn flooding, and the resulting turbidity in the water column. When restoring river sections with the placement of stones, gravel and sand, it must be considered where the substrate is taken from. The young mussels need food, and the food is related to organic material. The diet of wild young mussels is not known, but it is related to dark rich soil and well degraded detritus. What will be the diet for young mussels clearly differs in every individual river. So what should be the food for the young and adult mussels must be taken into consideration. Another important aspect is that different types of food provide different possibilities to reduce contamination of, for instance, heavy metals. Furthermore, when importing substrate from other parts of a river or land, it must be checked for natural levels of heavy metals as well for other contaminants. Most optimal is to use local material that has the same properties as the local good quality river substrate. Buffering of pH with lime or other calcium-rich material will possibly be important temporarily, i.e. during acidification from high nitrogen events. This is important in order to limit periodically high heavy metal bioavailability. It is also seen that as a result of its emissions the effect of large-scale industry can negatively influence rebuilding the recruitment measures of the freshwater pearl mussels. Under all circumstances, investment in cleaner production on local, regional and global scales seems to be important for successful restoration of freshwater pearl mussel populations.

For restoration, small measures may be carried out to manipulate river habitats in order to improve physical parameters. Even though, large-scale work is then needed for stabilizing nutrition and avoiding the creation and accumulation of poisonous components such as nitrite. Reduction and detoxification of heavy metals by the use of mineral rich materials might be needed in future to a greater extent due to the increased nitrogen compounds and acidification agents transported from far away. It also seems important to secure water reservoirs in peat and mires as well as ground water so as to secure water rinsing and the adjustment of river water temperature along with the mussel populations. Trees provide shade on the river, secure the banks of the river and stabilize the production of various plant species that later turn into detritus. The species composition in the vegetation zones influences the effect of rinsing surface soil water and halts the flow of materials and nutrients as well as pollutants into the river.

How to monitor: Monitoring must be long-term and should be followed up with all the indicator parameters at actual freshwater pearl mussel sites and key sites of the freshwater pearl mussel distribution along the river. Key sites can, for instance, be important recruitment areas, larger population density areas, and high-density host areas. The monitoring must also cover the important periods in the river such as the snow-melt period as well as other floods and droughts, and nonetheless important periods in freshwater pearl mussels annual cycle. Every river is unique and therefore it is necessary to make thoroughly designed individual monitoring plans.

Future research needs: More knowledge is needed to understand the cocktail (combined) effects and the added climate/temperature influence on the metabolic processes in the freshwater pearl mussel, hence the uptake and neutralization of heavy metals and other toxic components

in the mussel. In addition to such direct effects, there is also a variety of indirect environmental effects influencing freshwater pearl mussel population dynamics. The young mussels alter their diet and metabolism when changing from parasitic to microorganism-/detritus-eating to filter feeding. Primarily, more knowledge is needed of the diet of the different age groups of freshwater pearl mussels and their metabolism. The toxicity of different pollutants is not known under the variety of environmental parameters found in the north. One interesting aspect is the longevity of the freshwater pearl mussels, even in very harsh conditions. More knowledge is also need of the relationship between genome, environment and pollutants.

Appendix 1

100 METEORITE IMPACT ROCKS AND SITES
101 Impact melt, impact breccia
102 Impact site
200 CONTINENTAL SHELF
210 Cenozoic rocks
211 Sediment underlain by ocean-floor basalt
212 Sandstone, conglomerate, siltstone, shale, limestone
213 Olivine nephelinite (Palaeocene)
220 Mesozoic rocks
221 Sandstone, siltstone, shale, coal, marl, limestone
230 Neoproterozoic (and possibly Mesoproterozoic) and Palaeozoic rocks
231 Volcanic, sedimentary and intrusive rocks, undifferentiated (Permo-Carboniferous)
232 Limestone, marl, oil shale, shale, siltstone, sandstone (Middle Cambrian to Permian)
233 Sandstone, conglomerate, siltstone, shale (Vendian to Lower Cambrian, in part even Middle Cambrian)
234 Sandstone, conglomerate, siltstone, shale (Upper Riphean and possibly older)
240 Uppermost Palaeoproterozoic and Mesoproterozoic rocks
241 Dolerite, basalt (c. 1.27-1.22 Ga, in part c. 1.47 Ga)
242 Sandstone, conglomerate, siltstone, shale (Riphean, pre-1.27 Ga)
243 Granite, syenite, monzonite, gabbro, anorthosite (Mesoproterozoic)
244 Granite, syenite, monzonite, gabbro, anorthosite, quartz porphyry (Uppermost Palaeoproterozoic)
300 NEOPROTEROZOIC (AND POSSIBLY MESOPROTEROZOIC) AND PHANEROZOIC ROCKS OUTSIDE THE CALEDONIAN OROGENIC BELT
310 Cenozoic rocks
311 Sandstone, siltstone, shale, limestone
320 Mesozoic rocks
321 Basalt (Jurassic and Cretaceous)
322 Sandstone, siltstone, shale, coal, marl, limestone
330 Neoproterozoic (and possibly Mesoproterozoic) and Palaeozoic rocks
331 Granite, syenite, monzonite, nepheline syenite, foid-bearing monzosyenite, diorite, gabbro, pyroxenite (Permo-Carboniferous)
332 Latite porphyry, dolerite (Permo-Carboniferous)
333 Rhyolite, trachyte, latite, basalt, sandstone, conglomerate (Permo-Carboniferous)
334 Nepheline syenite, foidolite, carbonatite, melilitolite, pyroxenite, peridotite (Devonian)
335 Lamprophyre, melilitite, nephelinite, dolerite (Devonian)
336 Phonolite, trachyte, alkali basalt, picrite (Devonian)
337 Limestone, marl, coal, oil shale, shale, siltstone, sandstone, conglomerate (Middle Cambrian to Permian)
338 Sandstone, conglomerate, siltstone, shale (Vendian to Lower Cambrian)
339 Nepheline syenite, carbonatite, pyroxenite, alnöite (Vendian to Cambrian)
340 Dolerite (Vendian to Cambrian)
341 Sandstone, conglomerate, siltstone, shale (Upper Riphean and possibly older)
Archaean rocks
351 Area with kimberlite pipes
400 CALEDONIAN OROGENIC BELT
410 Neoautochthonous rocks
411 Sandstone and conglomerate (Devonian, in part uppermost Silurian)
420 Exotic and outboard terranes in Upper and Uppermost Allochthons
421 Granite, syenite, monzonite, granodiorite, tonalite, trondhjemite, diorite, dolerite including sheeted dyke complex, gabbro, ultramafic rock (Lower Palaeozoic)
422 Mica schist, paragneiss, marble, calc-silicate gneiss, quartzite, conglomerate, amphibolite in Uppermost Allochthon
423 Metagreywacke, phyllite, conglomerate, quartzite, limestone, felsic and mafic metavolcanic rocks in Upper Allochthon (Lower Palaeozoic)
430 Continent-ocean transition zone in Upper Allochthon
431 Metadolerite including sheeted dyke complex, amphibolite, gabbro, eclogite, ultramafic rock
432 Garnet-mica schist, quartzo-feldspathic schist, quartzite, marble, amphibolite
433 Metagranite (c. 1.65 Ga)
434 Anorthosite, gabbro, ultramafic rock and metamorphic equivalents
440 Continental margin in Middle Allochthon
441 Gabbro, ultramafic rock, nepheline syenite, carbonatite, granite (Neoproterozoic to Cambrian)
442 Dolerite (Neoproterozoic)
443 Feldspathic metasandstone, meta-arkose, quartzite, metagreywacke, marble, tillite (Neoproterozoic)
444 Granite, syenite, monzonite, tonalite and metamorphic equivalents (c. 1.70-0.90 Ga)
445 Gabbro, anorthosite and metamorphic equivalents (c. 1.70-0.90 Ga)
446 Felsic metavolcanic rock, metaporphyry
450 Continental margin (including platformal rocks) in Lower Allochthon and in windows with uncertain tectonostratigraphic status
451 Arkose, quartzite, greywacke, siltstone, shale, phyllite, limestone, dolomite, tillite
452 Granite, granodiorite, quartz monzonite, monzogabbro (1.00-0.90 Ga)
453 Gabbro, eclogite, ultramafic rock (in part c. 1.45 Ga)
454 Granite, granodiorite, tonalite and metamorphic equivalents (c. 1.70-1.51 Ga)
455 Felsic volcanic rock, porphyry
456 Mafic and intermediate volcanic rocks
457 Granite, granodiorite, syenite, monzonite and metamorphic equivalents (c. 2.20-1.70 Ga)
458 Gabbro, diorite, anorthosite, ultramafic rock and metamorphic equivalents (c. 2.20-1.70 Ga)
459 Mica gneiss, paragneiss, subordinate marble and graphite schist
460 Quartzite, metagreywacke, mica schist, minor mafic metavolcanic rock (c. 2.30 Ga)
461 Mafic metavolcanic rock, banded silicate-carbonate rock, serpentinite (c. 2.30 Ga)
462 Granite, granodiorite, tonalite and metamorphic equivalents
463 Migmatitic gneiss of granodioritic to dioritic composition
500 NEOPROTEROZOIC (TO MESOPROTEROZOIC) ROCKS
501 Gabbro, norite, anorthosite, monzogabbro, monzonite, quartz monzonite (c. 0.93-0.92 Ga)
502 Granite, pegmatite (c. 1.00-0.92 Ga)

600 MESOPROTEROZOIC (TO PALAEOPROTEROZOIC) ROCKS
601 Meta-arkose, metagreywacke, conglomerate, quartzite, marble, mafic and felsic metavolcanic rocks (younger than c. 1.27 Ga)
602 Granite, granodiorite, trondhjemite, monzonite, monzodiorite, diorite and metamorphic equivalents (c. 1.25-1.00 Ga)
603 Granite, syenite and metamorphic equivalents (c. 1.25-1.20 Ga)
604 Lamprophyre, lamproite (c. 1.23-1.15 Ga)
605 Dolerite (c. 1.27-1.22 Ga)
606 Granite, monzonite, syenite and metamorphic equivalents (c. 1.56-1.20 Ga)
607 Granite, quartz monzonite, syenite and metamorphic equivalents, in part hypersthene-bearing (c. 1.46-1.30 Ga)
608 Gabbro, pyroxenite, anorthosite, dolerite and metamorphic equivalents (c. 1.46-1.30 Ga)
609 Quartzite (c. 1.45 Ga or younger)
610 Sandstone, conglomerate, siltstone, shale (Riphean, pre-1.27 Ga)
611 Basalt, dolerite
612 Meta-andesite, metarhyolite, metasandstone, quartzite, conglomerate, amphibolite (c. 1.50 Ga)
613 Granite, quartz monzonite, syenite, nepheline syenite and metamorphic equivalents (c. 1.58-1.47 Ga)
614 Gabbro, pyroxenite, anorthosite, dolerite and metamorphic equivalents (c. 1.58-1.47 Ga)
615 Dolerite, metadolerite, amphibolite, gabbro, metagabbro, granophyre (c. 1.57-1.47 Ga)
616 Granite, granodiorite, monzonite, monzodiorite, diorite and metamorphic equivalents
617 Granodiorite, tonalite, granite, monzonite and metamorphic equivalents (c. 1.61-1.56 Ga), banded orthogneiss and paragneiss
618 Gabbro, diorite, ultramafic rock and metamorphic equivalents
619 Metagreywacke, quartzite, paragneiss, mafic metavolcanic rock (c. 1.60 Ga)
620 Quartzite, mica schist, mica gneiss, marble, hornblende gneiss, amphibolite
700 PALAEOPROTEROZOIC ROCKS (1.71-1.61 Ga and possibly older)
701 Quartz porphyry, basalt (c. 1.62 Ga)
702 Dolerite (c. 1.65-1.62 Ga)
703 Granite, monzonite, syenite (c. 1.65-1.62 Ga)
704 Gabbro, anorthosite, dolerite (c. 1.65-1.62 Ga)
705 Felsic metavolcanic rock (c. 1.66 and c. 1.61 Ga)
706 Granite, quartz monzonite, monzonite, quartz syenite and metamorphic equivalents (c. 1.71-1.66 Ga, in part older, possibly as old as c. 1.86 Ga)
707 Gabbro, diorite, dolerite, ultramafic rock and metamorphic equivalents (c. 1.71-1.66 Ga, in part possibly as old as c. 1.86 Ga)
708 Sandstone, conglomerate
709 Rhyolite, trachyte, trachydacite (c. 1.71-1.69 Ga)
710 Trachybasalt, basaltic trachyandesite, trachyandesite (c. 1.71-1.69 Ga)
711 Metagranite (c. 1.71-1.66 Ga)
712 Metagranodiorite, metatonalite (c. 1.71-1.66 Ga)
713 Gabbro, diorite, ultramafic rock and metamorphic equivalents
714 Granitic orthogneiss, fine- to medium-grained (c. 1.70 Ga or possibly older)
715 Felsic and mafic metavolcanic rocks, paragneiss, quartzite (c. 1.70 Ga or possibly older)
750 PALAEOPROTEROZOIC ROCKS (1.96-1.75 Ga)
751 Dolerite, gabbro, metagabbro (c. 1.77 Ga)
752 Lamprophyre (c. 1.84-1.77 Ga)
753 Dunite, peridotite, pyroxenite, gabbro, alkaline gabbro, alkaline granite, nepheline syenite, carbonatite
754 Rhyolite (c. 1.80-1.78 Ga), conglomerate, sandstone
755 Basaltic andesite, trachyte, trachydacite, dacite (c. 1.80-1.78 Ga)
756 Granite, granodiorite, quartz monzonite, monzonite, syenite and metamorphic equivalents, in part hypersthene-bearing (c. 1.86-1.84 Ga and c. 1.82-1.76 Ga)
757 Gabbro, diorite, ultramafic rock and metamorphic equivalents (c. 1.86-1.77 Ga)
758 Granodiorite, tonalite, granite and metamorphic equivalents (c. 1.86-1.84 Ga and c. 1.77 Ga)
759 Granite, pegmatite (c. 1.85-1.75 Ga)
760 Granodiorite, granite, pegmatite, subordinate gabbro (c. 1.86-1.85 Ga)
761 Red sandstone and mudstone, conglomerate, metasandstone, quartzite, phyllite, volcanic and metavolcanic rocks
762 Granite, granodiorite (c. 1.88-1.87 Ga)
763 Granite, monzonite, syenite, in part pyroxene-bearing (c. 1.88-1.87 Ga)
764 Gabbro, diorite, monzodiorite, ultramafic rock (c. 1.88-1.87 Ga)
765 Peridotite, pyroxenite, gabbro
766 Dolerite dyke complex (c. 1.88 Ga)
767 Mafic to intermediate volcanic and metavolcanic rocks (c. 1.88-1.86 Ga, in part possibly younger)
768 Felsic to intermediate volcanic and metavolcanic rocks (c. 1.88-1.86 Ga, in part possibly younger)
769 Granodiorite, tonalite, granite, monzonite, syenite and metamorphic equivalents, in part hypersthene-bearing (c. 1.91-1.88 Ga, in part as young as c. 1.84 Ga)
770 Gabbro, diorite, ultramafic rock and metamorphic equivalents (c. 1.91-1.88 Ga, in part as young as c. 1.84 Ga)
771 Quartzite, meta-arkose
772 Marble
773 Mafic metavolcanic rock (c. 1.90-1.88 Ga)
774 Felsic to intermediate metavolcanic rock (c. 1.90-1.88 Ga)
775 Metagreywacke, metasiltstone, metasandstone, mica schist, graphite- and/or sulphide-bearing schist, paragneiss, amphibolite intercalations (c. 1.95-1.87 Ga and possibly older)
776 Picrite, basalt, andesite and high-Mg andesite, metamorphosed
777 Andesite, dacite and rhyolite, metamorphosed
778 Mafic metavolcanic rock (c. 1.92-1.91 Ga)
779 Felsic to intermediate metavolcanic rock (c. 1.92-1.91 Ga)
780 Granodiorite, tonalite, granite, gabbro and metamorphic equivalents; alkaline gneiss (c. 1.96-1.91 Ga)
781 Mafic metavolcanic rock (c. 1.95 Ga and/or older)
782 Felsic metavolcanic rock (c. 1.95 Ga and/or older)
783 Ophiolite complex including serpentinite, gabbro, sheeted dykes, pillowed tholeiitic metabasalt, black schist, chert (c. 1.96-1.95 Ga)

800 LAPLAND-WHITE SEA GRANULITE BELT (rocks of uncertain age, in time range 2.30-1.90 Ga)
801 Anorthosite
802 Felsic to intermediate granulitic rock
803 Mafic to intermediate granulitic rock
804 Mafic granulitic rock, amphibolite
850 PALAEOPROTEROZOIC ROCKS (2.50-1.96 Ga)
860 Rock group 2.06-1.96 Ga
861 Mica schist, metagreywacke, black schist, conglomerate
862 Gabbro and dolerite, of variable ages
863 Ferrodolerite, monzodiorite
864 Tholeiitic basalt, rhyolite, chert, jasper, banded iron formation
865 Tholeiitic basalt, ferropicrite, picrite, peridotite, pyroxenite, gabbro, wehrlite/dolerite
866 Gabbro, peridotite
867 Komatiite, picrite, tholeiitic basalt
868 Black schist, carbonaceous quartzite, siltstone, shungitic rocks, dolostone, imestone, basalt, andesitic basalt, picrobasalt/dolerite
869 Tholeiitic basalt, arkosic sandstone, quartzite, greywacke, dolostone, black schist
870 Rock group 2.30-2.06 Ga
871 Dolostone, stromatolitic dolostone, arkosic sandstone, quartzite, siltstone, limestone, basalt
872 Trachybasalt, trachyandesite, tholeiitic basalt, picrite, dacite, quartzite, arkosic sandstone, dolostone, stromatolitic dolostone, jasper
873 Tholeiitic basalt, subordinate quartzite and conglomerate
874 Quartzite, mica schist, mica gneiss, conglomerate
880 Rock group 2.40-2.30 Ga
881 Basalt, high-Mg basalt, high-Mg andesite, dacite, komatiitic basalt/dolerite
882 Mica schist, conglomerate, gritstone, diamictite, arkosic sandstone, quartzite, tuffite
890 Rock group 2.50-2.40 Ga
891 Granite, quartz syenite, quartz monzonite, monzonite, charnockite
892 Layered intrusion: gabbro, gabbro-norite, anorthosite, dunite, peridotite, pyroxenite
893 Anorthosite, gabbro
894 Rhyolite, dacite, greywacke, conglomerate
895 Tholeiitic, komatiitic and andesitic basalt, andesite, dacite, peridotite, gabbro, siltstone, quartzite, arkosic sandstone
896 Basalt, andesite, komatiitic basalt, dacite, quartzite, arkosic sandstone
897 Graphite-bearing, garnet-kyanite-staurolite schist, sericite schist, quartzite, arkosic sandstone, conglomerate
900 ARCHAEAN ROCKS
910 Plutonic rocks and undifferentiated gneiss and migmatitic rock complexes
911 Granodiorite, granite, porphyritic granite (c. 2.60-2.50 Ga)
912 Carbonatite (c. 2.60 Ga)
913 Alkaline granite, alkaline syenite
914 Anorthosite, gabbro (c. 2.65 Ga)
915 Granite, pegmatite (c. 2.70-2.65 Ga)
916 Gabbro, monzodiorite, syenite, granodiorite (c. 2.74-2.65 Ga)
917 Granite, granodiorite, diorite, quartz diorite, porphyritic granite (c. 2.75-2.65 Ga)
918 Diorite, tonalite, granodiorite, trondhjemite, enderbite, charnockite (c. 3.00-2.74 Ga)
919 Tonalite-trondhjemite-granodiorite gneiss, quartzo-feldspathic gneiss, enderbite, migmatitic gneiss, with mafic and felsic enclaves (c. 3.20-2.65 Ga and possibly older)
920 Amphibolite - schist - gneiss belts.
921 High-Al mica schist, mica gneiss, hornblende gneiss with amphibolite enclaves
922 Mica schist and mica gneiss, migmatitic gneiss, amphibolite, banded iron formation
923 Amphibolite, amphibole gneiss
930 Volcanic-dominated greenstone belts (c. 3.20-2.75 Ga and possibly older)
931 Komatiite, basalt, andesite, dacite, rhyolite
932 Andesite, dacite, rhyolite, greywacke, arkosic sandstone, conglomerate, amphibolite, mica schist, locally banded iron formation
933 Tholeiitic, komatiitic and Fe-rich tholeiitic basalt, peridotite, gabbro, dacite, rhyolite, conglomerate

Annex D

Population genetic analyses of northern freshwater pearl mussel populations

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1 Introduction

1.1 Genetic diversity

Effective conservation approaches for endangered species such as the freshwater pearl mussel (*Margaritifera margaritifera)* require the integration of ecological and genetic information (Geist 2010). Low genetic diversity is a matter of concern, as it may reduce the ability of the species to adapt to changes in the environment. Therefore, maintaining genetic diversity has been identified as one of the key elements in successful conservation programmes (McNeely et al. 1990; Frankham et al. 2002). Knowing the genetic structure of *Margaritifera margaritifera* populations is an important baseline for conservation acts. Genetically diverse populations with high allelic richness (many variable copies of genes) should be of high priority both nationally and internationally. Populations having unique alleles, even though their diversity might be low, are also important as they may represent the only population worldwide with that particular allele. Genetic analyses may also reveal past migration routes and present gene flows between populations. Moreover, genetic results have an important diagnostic value – they can tell us whether the population suffers from inbreeding or whether it has been near extinction in the past (the "bottleneck effect"). To strengthen low density *M. margaritifera* populations or to restore extinct populations, different supportive breeding and planting projects have been carried out (Gum et al. 2011). Adult mussels, juveniles reared in captivity, or fish hosts with pearl mussel glochidia larvae have been introduced into target rivers. Knowledge of the genetic structure and genetic differentiation of *M. margaritifera* populations could be used to optimize introduction efforts, for example to increase genetic diversity or decrease inbreeding. In addition, the re-introduction of individuals from genetically incompatible sources breaking the locally developed genetic adaptations can be avoided.

In this study, two types of molecular data were used. First, we used mitochondrial DNA (mtDNA) sequences, which are widely utilized in population genetic analysis, and particularly in phylogeography (Avice et al. 1987). Second, we used microsatellites, which are short repeating nuclear DNA sequences used in mapping genomes, in forensic work and in conservation genetics (Ellegren 2004). To generate more reliable and comprehensive information about freshwater pearl mussel genetics based on markers under different evolutionary selection mechanisms, mitochondrial and microsatellite approaches were combined in this study.

Except for some Norwegian rivers (Karlsson & Larsen 2013), the River Luttojoki and the River Luiro (Geist and Kuehn 2008), no information on the genetic structure and differentiation of *M. margaritifera* populations in the present project area was available. Previous results by Geist and Kuehn (2008) indicate that the Rivers Luttojoki and Luiro are a hotspot of genetic diversity among European *M. margaritifera* populations.

Also, Swedish and Norwegian populations were found to have a surprisingly high genetic variability compared to central and southern European populations (Geist et al. 2010; Karlsson & Larsen 2013). Consequently, the following questions were addressed: Are other northern Fennoscandian pearl mussel populations also similarly genetically diverse? Are there differences between large rivers and small tributaries with respect to genetic diversity? Do some pearl mussel populations bear unique alleles? Do some populations show signals of low diversity, inbreeding or the bottleneck effect? Are some populations genetically more valuable than others? Are salmon rivers genetically richer than brown trout rivers? The aim of the present study is to shed light on these questions, and to interpret population genetic data in the context of information on the habitat and history of populations.

1.2 Some key terms in population genetics

The Hardy–Weinberg principle, or equilibrium states, that after one generation of random mating, single-locus genotype frequencies can be represented by a binomial or multinomial function of the allele frequencies (Hedrick 2005). In other words, it states that, in a large randomly breeding population (ideal panmictic population), allele frequencies will remain the same from generation to generation, assuming that there is no mutation, gene flow, selection or genetic drift. If genotype frequencies of the population deviate from Hardy–Weinberg predictions, it is evident that they are influenced by evolutionary forces such as genetic drift or gene flow (migration).

The number of alleles – also called as allelic diversity or allele richness or *A*, is simply a count of the number of alleles observed at a microsatellite locus in a population. Rarefaction is an approach to correct estimates of *A* for differences in sample size. Locus means a place on a chromosome, where a particular gene or DNA sequence is located, and an allele is a variant of the gene or DNA sequence at this place (locus). Similarly, the number of haplotypes, or haplotype richness, is a count of mitochondrial DNA haplotypes.

The diversity index for mtDNA haplotypes (h) is equivalent to the heterozygosity for diploid data (e.g. microsatellites). It is a probability that two randomly selected haplotypes are different in the sample (Nei 1987). High diversity index values indicate that the distribution of haplotypes/alleles is not dominated by one or a few haplotypes/alleles.

Heterozygosity is a measure of genetic variation within a population. Expected heterozygosity (H_E) or gene diversity is an expected heterozygosity under the Hardy–Weinberg equilibrium. High heterozygosity indicates high genetic variability, and vice versa. The observed level of heterozygosity (H_O) is compared to expected hetorozygosity (H_E). If the observed heterozygosity is lower than expected, it can be indicative of inbreeding. If heterozygosity is higher than expected, it might be caused, for example, by an isolate-breaking effect (the mixing of two previously isolated populations).

Differentiation measures, such as *F* coefficients (Wright 1965), are used to allocate the genetic variability to the total population level, subpopulations and individuals (Hedrick 2005). F_{ST} is a measure of the genetic differentiation between populations (e.g. the F_{ST} value 0.22 means that 22% variation occurs between populations and the rest of the variation occurs within the populations). F_{IS} is a measure of the deviation from the Hardy–Weinberg equilibrium within populations, where positive values indicate inbreeding.

2 Material and Methods

2.1 Mitochondrial DNA and haplotype data

Sampling

Samples were collected in the autumn of 2011 from 21 pearl mussel populations (Fig. 1) originating from five Finnish main drainage systems – the Rivers Iijoki (4 populations), Kemijoki (4 populations), Tenojoki (1 population), Torniojoki (3 populations), Luttojoki (the River Tuloma drainage, 5 populations), from two Norwegian main drainage systems Pasvik

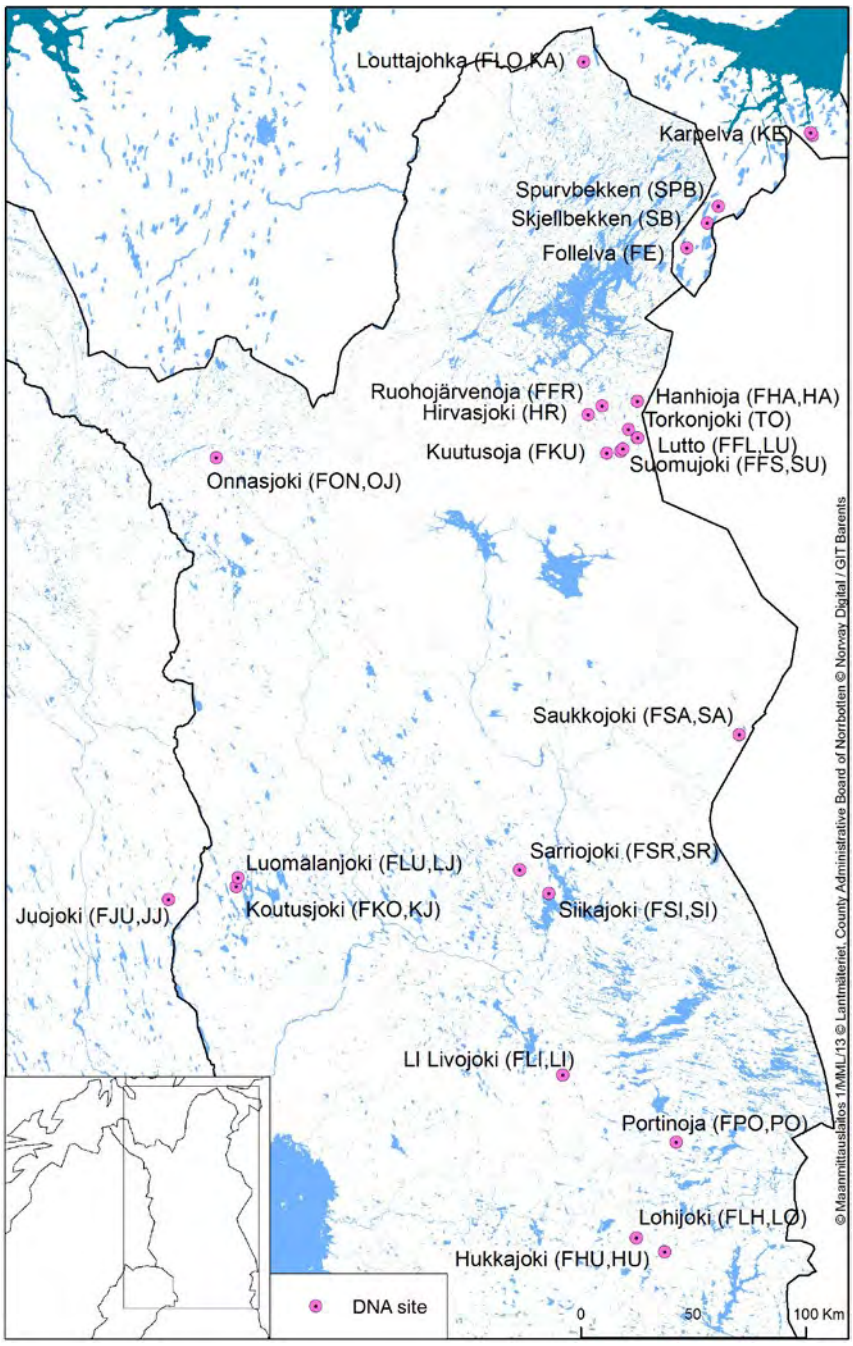

Figure 1. Freshwater pearl mussel DNA sample collection sites. After the name of the river, follows its code for microsatellite and mtDNA samples in parentheses. © Metsähallitus 2015, © National Land Survey of Finland 1/MML/15, ©Läntmäriet, County Administrative Board of Norrbotten, © Norway Digital / GIT Barents.

(3 populations) and Karpelva (1 population), a total of 622 individuals (Table 1).

The mussels were measured with a caliper for length and height. DNA-samples from the mantle tissue were taken by using a non-destructive mantle biopsy method (Berg et al. 1995; Karlsson et al. 2013a). After sampling, the mussels were returned alive into the river. Sample pieces were contained in 1.5 ml Eppendorf vials with absolute ethanol. The necessary sampling permits were granted by the environmental authorities.

DNA isolation and mtDNA COI *haplotype analyses*

Total DNA was extracted from mantle tissue using NucleoSpin Tissue-Kit (Macherey-Nagel), following the manufacturer's instructions for preparation of tissue material. Isolated total DNA was used to amplify the mitochondrial cytochrome oxidase subunit I (*COI*). The *COI* fragments were amplified by polymerase chain reaction (PCR) using the

Table 1. Sampling river, population size, host fish, date, sample size (N), GPS coordinates of sampling sites (WGS84) and main drainage of DNA samples. Host fish means the probable primary host fish species.

N. of the River	River	Population size	Host fish pop.	Sampling date	Samples	Drainage basin
1	Hanhioja	15,700	Trout	6.9.2011	30	Tuloma
2	Torkonjoki	7,200	Trout	7.9.2011	30	Tuloma
3	Hirvasjoki	4,600	Trout	8.9.8011	30	Tuloma
4	Luttojoki	150,000*	Salmon	9.9.2011	30	Tuloma
5	Suomujoki	133,600	Salmon	10.9.2011	30	Tuloma
6	Louttajohka	3,800	Trout	11.9.2011	25	Teno
7	Livojoki	5,300	Salmon	19.9.2011	30	Iijoki
8	Portinoja	n/a	Trout	20.9.2011	30	Iijoki
9	Lohijoki	n/a	Trout	21.9.2011	30	Iijoki
10	Hukkajoki	n/a	Trout	21.9.2011	30	Iijoki
11	Saukkojoki	27,200	Trout	10.10.2011	30	Kemijoki
12	Sarriojoki	n/a	Trout	10.10.2011	30	Kemijoki
13	Siikajoki	42,800	Trout	10.10.2011	30	Kemijoki
14	Luomalanjoki	n/a	Trout	11.10.2011	30	Torniojoki
15	Koutusjoki	131,500	Trout	11.10.2011	30	Torniojoki
16	Juojoki	42,000	Trout	11.10.2011	30	Torniojoki
17	Onnasjoki	14,500	Trout	12.10.2011	30	Kemijoki
18	Føllelva	14,000	Trout	1.9.2011	28	Pasvik
19	Skjellbekken	30,000	Trout	1.9.2011	30	Pasvik
20	Spurvbekken	20,000	Trout	1.9.2011	29	Pasvik
21(a)	Karpelva(a)	700,000	Salmon	1.9.2011	15	Karpelv
21(b)	Karpelva(b)	same river	same river	1.9.2011	15	Karpelv
Total sample size					**622**	

following primers: 5'-GGTCAACAAATCATAAAGATATTGG-3' and 5'-TCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). The PCR mix in a total volume of 20.0 µL, contained the following components: 50 ng of genomic DNA, 1.0 µM of both primers, 0.2 mM of each dNTP, 2.0 mM $MgCl_2$, 1 × PCR buffer ($(NH_4)_2SO_4$, Fermentas), 0.4 U Taq DNA Polymerase (Fermentas). PCR was carried out using a S1000 Thermal Cycler (Bio-Rad) under the following conditions: 94 °C (3 min), 34 cycles with denaturation at 94 °C (30 s), annealing at 50 °C (1 min), extension at 72 °C (1 min) and a final extension at 72 °C (7 min). DNA electrophoresis on a 1% agarose gel stained with SYBR Green I (Life Technologies) was used to verify that the PCR amplifications were successful. Before sequencing, the amplified *COI* fragments were purified by using the Exo-SAP method. Sequencing PCR was carried out by using the ABI PRISM BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) following the manufacturer's instructions. Purified sequencing PCR products were sequenced by using the 3730 DNA Analyzer (Applied Biosystems). The raw *COI* sequence data was edited and aligned with Sequencing Analysis Software 6 (Applied Biosystems), MEGA 5.2 software (Tamura et al. 2011) and ClustalW (Thompson et al. 1994) program.

Statistical and population genetic analyses

The most suitable evolutionary model for the sequence data was determined by using MEGA 5.2 software. According to the results, this was the HKY85 model (Hasegawa et al. 1985). It was

used to calculate the genetic distances between haplotypes in PAUP 4.0b10 program (Swofford 2001). DnaSP v5 software package (Librado and Rozas 2009) was used to calculate the number of haplotypes (H), haplotype frequencies and haplotype diversities (h). The sample size corrected haplotype richness (H_R) and private haplotype richness was calculated by using the rarefaction method and HP-RARE 1.0 program (Kalinowski 2005). Genetic structure and differentiation of populations were analysed by using an analysis of molecular variance (AMOVA) with the Arlequin ver. 3.5.1.2 software package (Excoffier et al. 2005). Populations were divided into different groups by their drainage system and by their host fish stock (salmon vs. brown trout rivers). The genetic distance matrix based on the HKY85 model was used to compute the AMOVA and population pairwise F_{ST} values and significances were tested by using 1000 permutations. The effect of geographical distance on the differentiation between populations was examined using the Mantel test (Mantel 1967) with the Arlequin software package. The population pairwise F_{ST} values were used to construct a neighbor-joining (NJ) phenogram (Saitou and Nei 1987) in MEGA 5.2 software. Mean haplotype richness and diversity of *M. margaritifera* from brown trout and salmon rivers were compared using analysis of variance (ANOVA) with population-specific values as the statistical unit. The dependence of observed mtDNA richness (H) on mussel population size (N) and fish species was studied using regression analysis. Asymptotic models

$$H = 1 / (1 / a + b / N) \quad (1)$$

$$H = 1 / (1 / (a + c * D_{Species}) + b / N), \quad (2)$$

where a, b and c are model parameters and $D_{Species}$ = dichotomous dummy-variable (0 = brown trout, 1 = salmon), were fitted to the data by least squares method. Thus, it was assumed for simplicity that both species had equal value of parameter b.

These two nested models were compared using F-test.

Furthermore, association between mitochondrial DNA results (e.g. haplotype richness) and microsatellite data (e.g. allelic richness) were studied using correlation analysis.

2.2 Genotypic data - microsatellite data

Sampling/specimens

A total of 433 individuals from 17 pearl mussel populations (Fig. 1) originating from five Finnish main drainage systems – the Rivers Iijoki (4 populations), Kemijoki (4 populations), Tenojoki (1 population), Torniojoki (3 populations) and Luttojoki (the River Tuloma drainage, 5 populations) – were included in the microsatellite study. The necessary sampling permits were granted by the environmental authorities. For comparison and for statistical analysis (neighbor-joining (NJ) phenogram), two central European populations from the Elbe catchment (Wolfsbach, Zinnbach) were also included (Table 7). This procedure is customary in order to root a phenogram.

DNA isolation and microsatellite analyses

As previously for the mtDNA, total DNA was extracted from mantle tissue using NucleoSpin Tissue-Kit (Macherey-Nagel). Nine microsatellite loci (MarMa2671, MarMa3050, MarMa3621, MarMa4143, MarMa4322, MarMa4726, MarMa5167, MarMa5280 and MarMa5023) were used for genetic analyses as described in Geist et al. (2003) and Geist and Kuehn (2005). Polymerase Chain Reactions (PCRs) were performed in a total volume of 12.5 µL with the following components: 25 ng of genomic DNA, 200 nM of each primer, 0.2 mM of each dNTP, 3 mM MgCl2 (2mM MgCl2 for locus MarMa5280), 1 × PCR buffer (10 mM Tris-HCl, 50 mM KCl, 0.08% Nonidet P40), and 0.25 U Taq DNA Polymerase (Qbiogene). The forward primers were labelled with the fluorescent dye Cy5. PCR was carried out on a Mastercycler gradient thermal cycler (Eppendorf) under conditions as described by Geist et al. (2003) and Geist and Kuehn (2005). PCR products were separated on 5% denaturing 19:1 acrylamide:bisacrylamide gels on an ALFexpressII DNA analyser and scored with ALLELELINKS 1.02 software (Amersham Parmacia Biotech). Electrophoresis was carried out with two internal standards in each lane. Additionally, an external standard and a previously sequenced reference sample were included

on each gel in order to ensure exact scoring and to facilitate cross-referencing among gels.

Statistical and population genetic analyses

GENEPOP version 4.0 (Rousset 2008) was used to calculate allele frequencies, average allele numbers per locus (*A*), expected and observed heterozygosities (H_E, H_O), to test the genotypic distribution for conformance with Hardy–Weinberg (*HW*) expectations, to test the loci for genotypic disequilibrium and to estimate the genetic differentiation (F_{ST} according to Weir and Cockerham 1984) between pairs of populations. Tests for significant population differentiation among all pairs of populations were performed with the same software using 100,000 iterations and 1,000 de-memorization steps (Raymond and Rousset 1995). Allelic richness (A_R) as a standardized measure of the number of alleles per locus corrected by the sample size was calculated with the software FSTAT version 2.9.3 (Goudet 2001). Genetic distances between populations were estimated using Nei D_A genetic distance (Nei et al. 1983) as implemented in the DISPAN program (Ota 1993). The resulting distance matrix was used to construct a neighbor-joining (NJ) phenogram (Saitou and Nei 1987) in MEGA version 6 (Tamura et al. 2013). Bootstrap values were calculated by generating 1,000 distance matrices with DISPAN (Ota 1993).

3 Results

3.1 Mitochondrial data

A total of 609 *COI* sequences were obtained from the 622 mantle tissue samples. Lengths of edited *COI* sequences were 658 base pairs. There were 18 variable nucleotide positions and haplotypes (Table 2). The open reading frame started from the 2/658 nucleotide position. There was one non-synonymous nucleotide substitution at the 482 nucleotide position, which changed the amino acid Alanine to Isoleucine.

Genetic diversity

Haplotype frequencies in populations are presented in Table 3 and relative haplotype frequencies in Figure 2. The number of haplotypes varied in Finland from one in the River Hirvasjoki to 8 in the River Luttojoki. Both rivers belong to the Tuloma drainage system. In Norway, the number of haplotypes varied between 3 (Føllelva) and 10 (Karpelva). Haplotype frequencies ranged in the whole mtDNA data from one to 169.The most frequent haplotypes were HT8 with 162 individuals and HT2 with 139 individuals. These were also the geographically most widespread haplotypes.

Seven of the haplotypes were 'private', i.e. unique and restricted to certain populations (Tables 3 and 4). HT20 and HT 21 were present only in the River Luttojoki, HT23 only in the River Livojoki and HT24 only in the River Koutusjoki, while haplotypes HT25, HT26 and HT27 were found only in populations of Onnasjoki, Karpelva and Skjellbekken, respectively. Haplotype richness (the number of observed haplotypes per population, H) ranged from 1 in the River Hirvasjoki to 10 in the River Karpelva. The calculated, expected haplotype richness (H_R) varied between 1.0 (the River Hirvasjoki) and 9.42 (the River Karpelva, Norway). In Finland the highest expected haplotype richness was observed in the River Luttojoki (7.12) of the Tuloma drainage. The haplotype diversity index (h) was highest in the River Karpelva, 0.884. Haplotype diversity index in Finnish drainage systems was highest in the River Koutusjoki (0.828) of the Torniojoki drainage system. Within the Tuloma drainage system haplotype diversity varied strongly, with a very high value in the River Luttojoki and a very low value in the River Hirvasjoki (Table 4).

Genetic differentiation

When studied with AMOVA there was no noticeable genetic differentiation between different drainage systems or between salmon and brown trout rivers. Hierarchical AMOVA revealed that 1% of the genetic variation was among drainage systems, 31.04% among populations within drainages, and 69.95% within populations (Table 5).

When populations were divided into salmon or brown trout rivers, 3.85% of the genetic variation was among different host fish rivers (F_{CT} = 0.038, P > 0.05). Population pairwise F_{ST} values,

Table 2. Variable nucleotide positions and their nucleotides between different *COI* haplotype sequences.

	Variable nucleotide positions (18/658)																	
Haplotypes	7	22	34	82	110	115	190	205	244	347	370	385	482	511	562	571	583	634
HT1	G	C	C	A	T	C	C	C	T	T	T	C	G	A	A	T	G	T
HT2	G	C	C	A	T	C	C	C	T	T	T	C	G	A	A	T	A	T
HT3	G	C	C	A	C	C	C	C	T	T	T	C	G	A	A	T	A	T
HT4	G	C	C	A	T	C	C	C	T	T	T	C	G	G	A	T	A	T
HT5	A	C	C	A	T	C	C	C	T	T	T	C	G	A	A	T	A	T
HT7	G	C	C	A	T	C	C	C	T	C	T	C	G	A	A	C	A	T
HT8	G	C	C	G	T	C	C	C	T	T	T	C	G	A	A	T	A	T
HT9	G	C	C	G	T	C	C	C	T	T	T	C	G	A	A	T	A	C
HT11	G	C	T	G	T	C	C	C	T	T	T	C	G	A	A	T	A	T
HT12	G	C	T	A	T	C	C	C	A	T	C	C	G	A	A	T	A	T
HT20	G	C	T	G	T	C	T	T	T	T	T	C	G	A	A	T	A	T
HT21	A	C	C	A	C	C	C	C	T	T	T	C	G	A	A	T	A	T
HT22	G	C	C	A	T	C	C	C	T	T	T	C	G	A	A	T	A	C
HT23	G	C	C	A	T	C	C	C	T	T	T	T	G	A	A	T	A	C
HT24	G	C	C	A	T	T	C	C	T	T	T	C	G	A	A	C	A	T
HT25	G	C	C	A	T	C	C	C	T	T	T	C	A*	A	A	T	G	T
HT26	A	C	C	A	T	C	C	C	T	T	T	C	G	A	G	T	A	T
HT27	G	T	C	A	T	C	C	C	T	T	T	C	G	A	A	T	A	T

(*) non-synonymous nucliotide substitution, Alanine to Isoleucine

Table 3. Haplotype frequencies in different populations of *M. margaritifera*. For haplotype details see Table 2. N = Sample size, A = Number of haplotypes, B = Number on unique haplotypes.

	Haplotypes																			
Population	HT1	HT2	HT3	HT4	HT5	HT7	HT8	HT9	HT11	HT12	HT20	HT21	HT22	HT23	HT24	HT25	HT26	HT27	N	A	B
Hanhioja							24		4										28	2	
Torkonjoki		3	8	1			10		8										30	5	
Hirvasjoki		25																	25	1	
Luttojoki		2	1	2	21	1	1				1	1							30	8	2
Suomujoki		1		4	22	1	2												30	5	
Louttajohka		4		6	8		1		3	2			1						25	7	
Livojoki	2	4				1	21							2					30	5	1
Portinoja	2	17					11												30	3	
Lohijoki		15	1			4	1	9											30	5	
Hukkajoki	5	2				2	21												30	4	
Saukkojoki		3			1	20	6												30	4	
Sarriojoki		3				3	17			7									30	4	
Siikajoki		13			2	1	8	2		4									30	6	
Luomalanjoki						9	21												30	2	
Koutusjoki	8	5			4	7	5								1				30	6	1
Juojoki	14	2	11										3						30	4	
Onnasjoki	12	11	5		1											1			30	5	1
Føllelva		18			5		4												27	3	
Skjellbekken							5						10					12	27	3	1
Spurvbekken		6	1		19		2												28	4	
Karpelva	1	5	2	5	6	1	2		5	1							1		29	10	1
Total	44	139	29	18	89	50	162	11	20	14	1	1	14	2	1	1	1	12	609		7

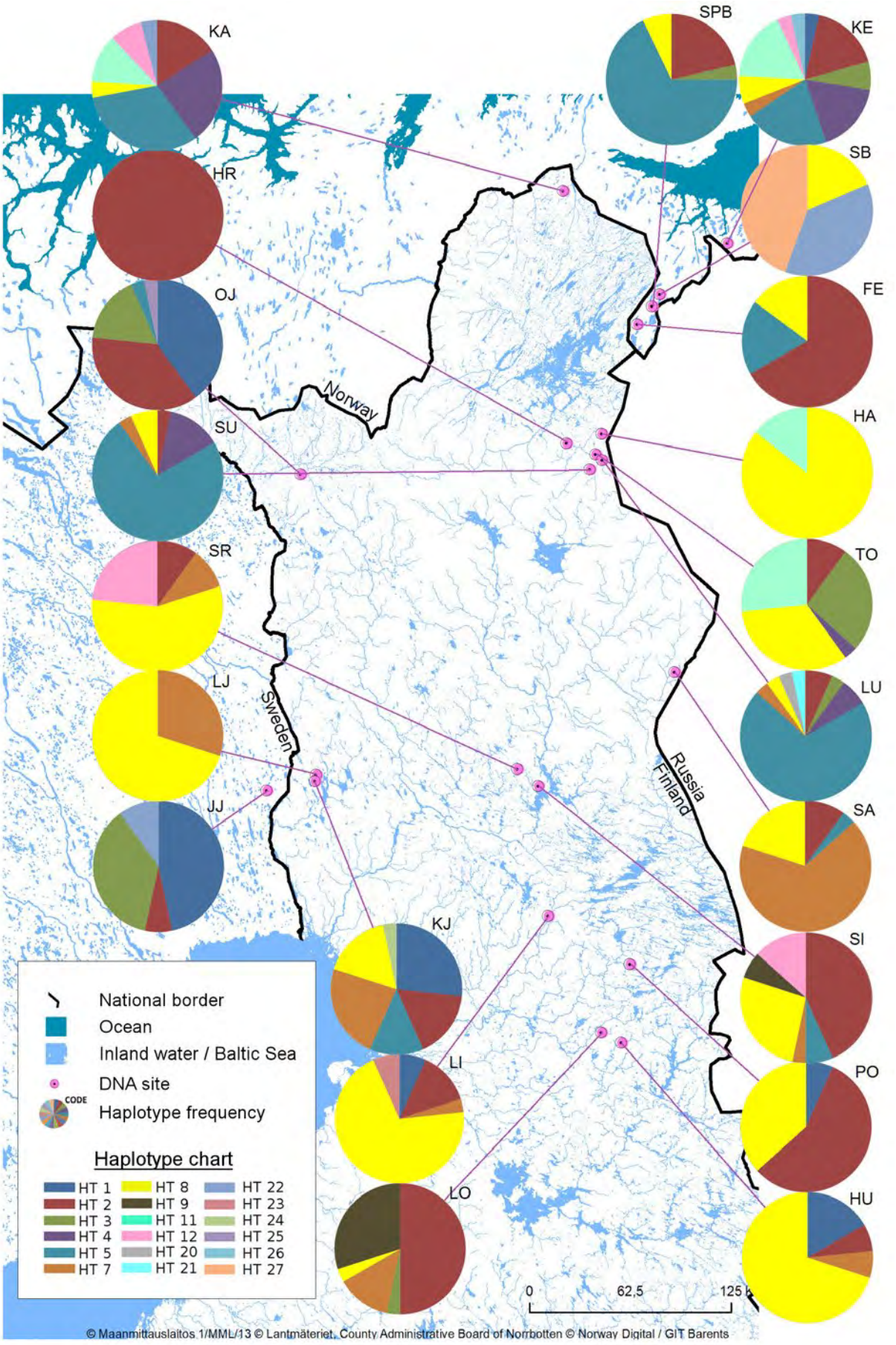

Figure 2. Relative haplotype frequencies in different *M. margaritifera* populations. © Metsähallitus 2015, © National Land Survey of Finland 1/MML/15, ©Läntmäriet, County Administrative Board of Norrbotten, © Norway Digital / GIT Barents.

Table 4. Haplotype diversity and richness in different populations of *M. margaritifera*. N = Sample size, H = Number of haplotypes, H_R (N = 25) = Haplotype richness, when sample size is 25, h = Haplotype diversity.

Population	Host fish pop.	N	H	H_R (N = 25)	Private H	h (S.D)	Drainage
Hanhioja	Trout	28	2	2		0.254 (0.095)	Tuloma
Torkonjoki	Trout	30	5	4.83		0.761 (0.035)	Tuloma
Hirvasjoki	Trout	25	1	1			Tuloma
Luttojoki	Salmon	30	8	7.12	2 (1.67)	0.513 (0.110)	Tuloma
Suomujoki	Salmon	30	5	4.64		0.453 (0.105)	Tuloma
Louttajohka	Trout	25	7	7		0.823 (0.044)	Teno
Livojoki	Salmon	30	5	4.79	1 (0.98)	0.499 (0.103)	Iijoki
Portinoja	Trout	30	3	2.98		0.559 (0.056)	Iijoki
Lohijoki	Trout	30	5	4.67		0.662 (0.061)	Iijoki
Hukkajoki	Trout	30	4	3.95		0.490 (0.098)	Iijoki
Saukkojoki	Trout	30	4	3.83		0.522 (0.091)	Kemijoki
Sarriojoki	Trout	30	4	4		0.625 (0.076)	Kemijoki
Siikajoki	Trout	30	6	5.79		0.738 (0.057)	Kemijoki
Onnasjoki	Trout	30	5	4.67	1 (0.83)	0.699 (0.046)	Kemijoki
Luomalanjoki	Trout	30	2	2		0.434 (0.070)	Torniojoki
Koutusjoki	Trout	30	6	5.83	1 (0.83)	0.828 (0.028)	Torniojoki
Juojoki	Trout	30	4	3.97		0.655 (0.053)	Torniojoki
Føllelva	Trout	27	3	3		0.519 (0.093)	Pasvik
Skjellbekken	Trout	27	3	3	1	0.655 (0.044)	Pasvik
Spurvbekken	Trout	28	4	3.88		0.505 (0.094)	Pasvik
Karpelva	Salmon	29	10	9.42	1 (0.86)	0.884 (0.028)	Karpelv

Table 5. Analysis of molecular variance (AMOVA), genetic structure and fixation indices between groups based on five Finnish and two Norwegian main drainage systems.

F_{SC} = test by permuting haplotypes among populations within drainages.
F_{ST} = test by permuting haplotypes among populations and among drainages.
F_{CT} = test by permuting whole populations among drainages.

Source of variation	d.f.	Sum of squares	Variance of components	Percentage of variation
Among drainages	6	43.104	0.00816 Va	0.99
Among populations within drainages	14	112.669	0.25579 Vb	31.04
Within populations	588	339.015	0.57656 Vc	69.95
Total	608	494.788	0.82418	
Fixation	**Indices**		**P-values**	
F_{SC}	0.30731		P < 0,001	
F_{ST}	0.30045		P < 0,001	
F_{CT}	0.00990		P > 0,05	

which indicate differentiation between populations ranged from 0.00 (between Luttojoki and Karpelva, among others) to 0.88 (between Hanhioja and Hirvasjoki). 94% of differences in all pairwise comparisons between populations were significant (P < 0.05). Only in 11 population pairs were the pairwise F_{ST} values not statistically significant (Table 6). The NJ phenogram (Fig. 3) was made to visualize the genetic relationships between populations based on the previously presented population pairwise F_{ST} values in the Table 6. The results of the Mantel test (r = -0.041 P = 0.662) confirmed that there was no isolation by distance of the populations.

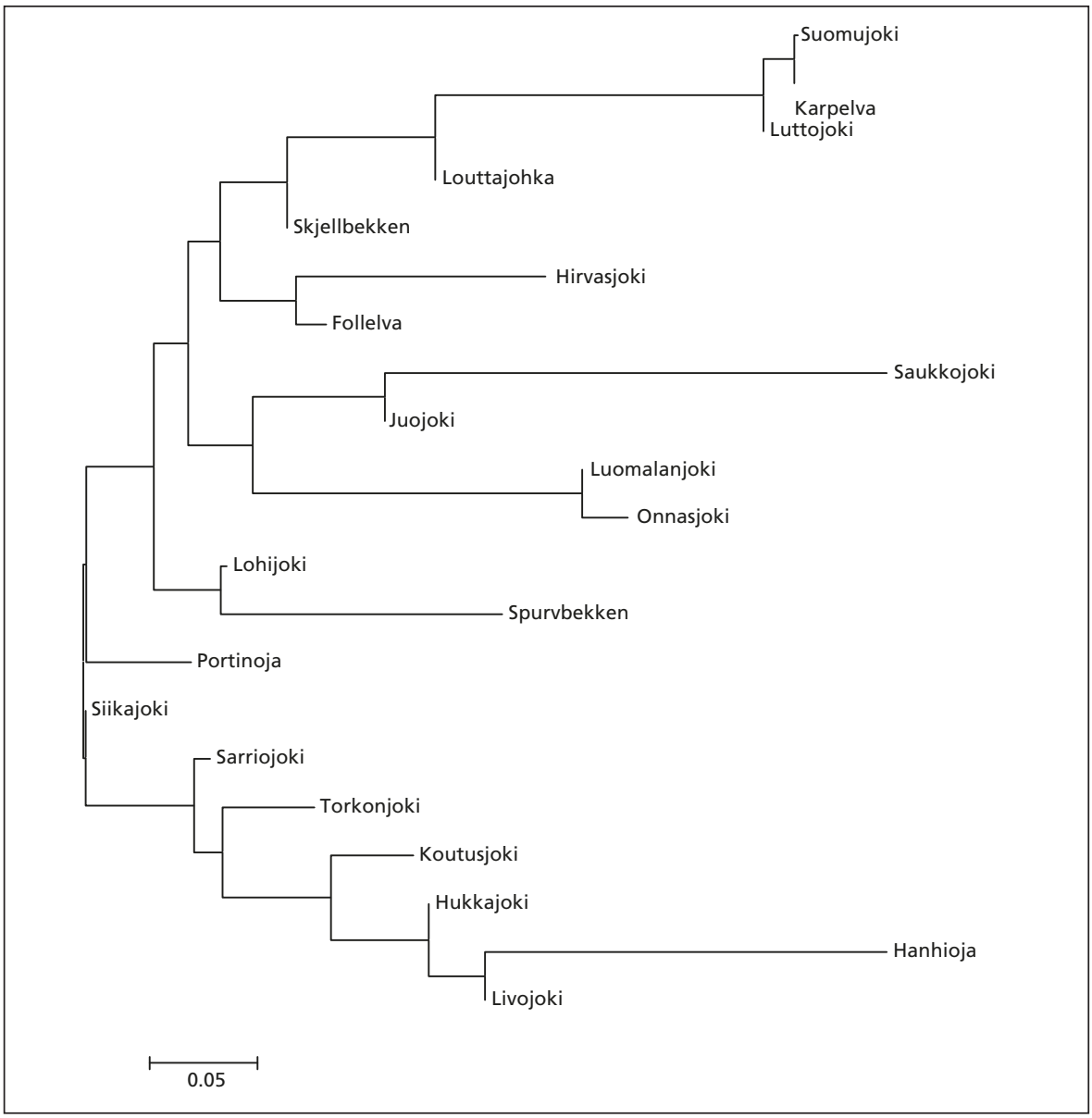

Figure 3. Neighbor-joining (NJ) phenogram based on pairwise F_{ST} values based on mtDNA haplotype data for Finnish, Norwegian and Swedish freshwater pearl mussel populations.

Thus, genetic differentiation between populations was not related to geographical distance.

When the population-wise averages were used as the statistical unit, both the mean observed haplotype richness and mean expected haplotype richness were higher in salmon rivers (n = 4) than in brown trout rivers (n = 17). The observed haplotype numbers (± standard error of mean) were on average 7.0 ±1.2 and 4.0 ± 0.4 in salmon and brown trout rivers, respectively, the difference being statistically significant (ANOVA, $F_{1,20}$ = 9.547, p = 0.006). Expected mean haplotype numbers were 6.5 ±1.1 and 3.9 ± 0.4 (ANOVA, $F_{1,20}$ = 9.547, p = 0.011), respectively. In the haplotype diversity index such a difference was not found. The mean diversity index for salmon (n = 4) and brown trout rivers (n = 16) was 0.59 and 0.61, respectively (ANOVA, $F_{1,19}$ = 0.053, p = 0.820).

It was possible to analyse the association between *M. margaritifera* population size and population genetic parameters for 15 rivers – in these rivers, the estimated number of mussels (census) was available and 11 of them were brown trout rivers and the rest were salmon rivers, i.e. the available host fish is brown trout ($D_{Species}$ = 0) or salmon ($D_{Species}$ = 1), respectively. Even in certain small populations, haplotype richness was almost at the same level as in the large populations. The model 2 (see Material and methods)

Table 6. Population pairwise F_{ST} values based on mtDNA haplotype data (significant values ($P < 0.05$) are marked as +) for Finnish, Norwegian and Swedish freshwater pearl mussel populations.

Drainage	Population	Code	HA	TO	HR	LU	SU	KA	LI	PO	LO	HU	SA	SR	SI	LJ	KJ	JJ	OJ	FE	SB	SPB	KE
Tuloma	Hanhioja	HA	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Torkonjoki	TO	0.21	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Hirvasjoki	HR	0.88	0.39	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Luttojoki	LU	0.67	0.41	0.46	0.00	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	Suomujoki	SU	0.71	0.45	0.52	0.00	0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Teno	Louttajohka	KA	0.46	0.19	0.18	0.13	0.13	0.00	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Iijoki	Livojoki	LI	0.15	0.10	0.48	0.47	0.50	0.26	0.00	+	+	-	+	+	+	+	+	+	+	+	+	+	+
	Portinoja	PO	0.48	0.15	0.28	0.42	0.45	0.16	0.11	0.00	+	+	+	+	-	+	+	+	+	+	+	+	+
	Lohijoki	LO	0.39	0.16	0.21	0.35	0.37	0.15	0.13	0.09	0.00	+	+	+	+	+	+	+	+	+	+	+	+
	Hukkajoki	HU	0.17	0.11	0.49	0.47	0.50	0.27	0.00	0.12	0.16	0.00	+	+	+	+	-	+	+	+	+	+	+
Kemijoki	Saukkojoki	SA	0.65	046	0.56	0.51	0.54	0.40	0.48	0.48	0.31	0.46	0.00	+	+	+	+	+	+	+	+	+	+
	Sarriojoki	SR	0.20	0.08	0.30	0.37	0.39	0.14	0.09	0.12	0.13	0.10	0.35	0.00	-	+	+	+	+	+	+	+	+
	Siikajoki	SI	0.33	0.10	0.14	0.28	0.31	0.06	0.11	0.03	0.05	0.13	0.37	0.02	0.00	+	+	+	+	+	-	+	+
Torniojoki	Luomalanjoki	LJ	0.67	0.34	0.28	0.39	0.43	0.22	0.40	0.26	0.25	0.37	0.50	0.32	0.22	0.00	+	+	-	+	+	+	+
	Koutusjoki	KJ	0.25	0.18	0.47	0.46	0.49	0.29	0.09	0.22	0.15	0.08	0.27	0.11	0.17	0.43	0.00	+	+	+	+	+	+
	Juojoki	JJ	0.46	0.24	0.18	0.26	0.28	0.13	0.23	0.13	0.10	0.20	0.20	0.17	0.11	0.12	0.18	0.00	+	+	+	+	+
Kemijoki	Onnasjoki	OJ	0.65	0.33	0.34	0.42	0.46	0.27	0.41	0.31	0.26	0.39	0.50	0.34	0.26	0.02	0.44	0.17	0.00	+	+	+	+
Pasvik	Føllelva	FE	0.65	0.26	0.13	0.25	0.29	0.07	0.30	0.10	0.13	0.31	0.48	0.21	0.06	0.24	0.34	0.12	0.30	0.00	-	+	+
	Skjellbekken	SB	0.37	0.11	0.13	0.15	0.16	0.00	0.18	0.08	0.10	0.18	0.35	0.10	0.03	0.17	0.21	0.09	0.22	0.04	0.00	+	+
	Spurvbekken	SPB	0.56	0.31	0.33	0.41	0.44	0.23	0.32	0.26	0.13	0.34	0.47	0.27	0.18	0.33	0.36	0.24	0.33	0.26	0.20	0.00	+
Karpelv	Karpelva	KE	0.74	0.45	0.56	0.00	0.00	0.14	0.51	0.46	0.37	0.51	0.56	0.38	0.29	0.43	0.50	0.27	0.45	0.27	0.16	0.44	0.00

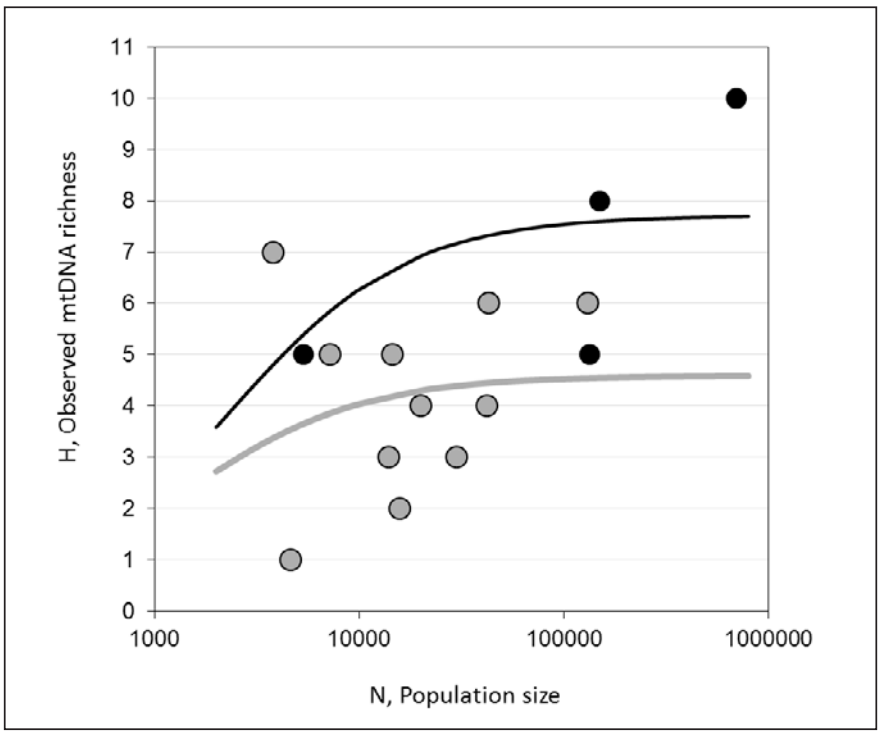

Figure 4. The fit of the model H = 1 / (1 / (4.59 + 3.12 * $D_{Species}$) + 299 / N) to the data for different M. margaritifera populations. Grey curve and symbols = main host fish brown trout ($D_{Species}$ = 0), black curve and symbols = main host fish salmon ($D_{Species}$ = 1). NOTE: logarithmic x axis.

incorporating the effects of both population size and species on mtDNA richness

H = 1 / (1 / (4.59 + 3.12 * $D_{Species}$) + 299 / N)
S.E. 0.83 1.34 317

gave a significantly better fit to the data than the model 1 without the species effect (the parameter c significantly different from 0 with risk p=0.037, the significance between difference between the fit of models 1 and 2 F=4.93, p=0.046) (Fig. 4). The asymptotic level of richness for salmon was estimated to be about 3 units higher than that for brown trout.

3.2 Microsatellite data

Linkage and Hardy–Weinberg equilibrium

The test for genotypic disequilibrium for each pair of the nine microsatellite loci over all populations showed one significant value (P < 0.0001) for 36 comparisons (two significant values are expected by chance at the 5% level). After Bonferroni correction for multiple tests, none of the combinations remained significant at the experimental level (P < 0.00138). Generally, this test implies that the genotypes of the loci used in this study segregated independently.

Six populations out of 19 displayed significant deviations from the expected HW proportions after applying sequential Bonferroni correction (see Table 7). These deviations are not systematic, occur at different loci (MarMa3621, MarMa4726, MarMa5167 and MarMa4143) and in different populations with a maximum of two deviations per population (the River Portinoja and the River Luomalanjoki)

Genetic diversity and genetic differentiation

An average of 4.7 alleles (standard deviation SD = 2.9) were observed for the nine microsatellite loci used in this study. The number of alleles per locus ranged from one at locus MarMa5280 which was monomorphic in all populations analysed to a maximum of 24 different alleles at locus MarMa3621. Mean allelic richness (A_R) varied between 1.9 (the River Wolfsbach, Germany) and 6.6 (the River Livojoki, Finland). Among the Finnish populations, the highest mean allelic richness was, thus, in the River Livojoki, while the lowest richness was found in the River Hanhioja (A_R =2.7). The average number of alleles (A) and mean allelic richness (A_R) varied strongly between populations within Finnish drainage systems, and was highest for

Table 7 Main Drainage, river, population code, country, sample size (N), GPS coordinates of sampling sites, average number of alleles per locus (A), mean allelic richness per population (A_R), expected (H_E) and observed (H_O) heterozygosity, F_{IS} value per population and result of Hardy-Weinberg probability test (HW); the Elbe populations (DWB and DZI) were used as central European outgroups (based on microsatellite data).

Main Drainage	River	Population Code	Country	Sample Size	GPS latitude	GPS longitude	A	A_R	H_E	H_O	F_{IS}	HW
Elbe	Wolfsbach	DWB	D	24	50.31677	12.12797	1.9	1.9	0.254	0.245	0.034	n.s
	Zinnbach	DZI	D	26	50.29544	12.12205	2.9	2.8	0.381	0.372	0.026	n.s
Iijoki	Lohijoki	FLH	FIN	25	65.26140	28.20848	5.6	5.4	0.579	0.564	0.026	n.s
	Hukkajoki	FHU	FIN	25	65.20300	28.47395	4.1	4.0	0.473	0.453	0.042	n.s
	Livojoki	FLI	FIN	25	65.91576	27.51852	6.9	6.6	0.535	0.516	0.037	***
	Portinoja	FPO	FIN	25	65.63980	28.61182	3.8	3.7	0.466	0.427	0.087	***
Kemijoki	Sarriojoki	FSR	FIN	25	66.73497	27.10295	3.8	3.7	0.380	0.360	0.054	n.s
	Saukkojoki	FSA	FIN	25	67.25679	29.36691	6.0	5.8	0.553	0.560	-0.013	n.s
	Siikajoki	FSI	FIN	25	66.63916	27.39588	5.3	5.0	0.445	0.453	-0.019	n.s
	Onnasjoki	FON	FIN	25	68.34746	23.83195	4.3	4.3	0.511	0.469	0.084	n.s
Teno	Louttajohka	FLO	FIN	25	69.95461	27.85942	5.9	5.7	0.584	0.521	0.109	***
Tornionjoki	Juojoki	FJU	S	25	66.57842	23.58036	5.3	5.2	0.565	0.551	0.024	n.s
	Koutusjoki	FKO	FIN	25	66.64458	24.25334	6.1	5.9	0.535	0.551	-0.031	n.s
	Luomalanjoki	FLU	FIN	25	66.67938	24.26650	5.4	5.3	0.565	0.528	0.066	***
Tuloma	Hanhioja	FHA	FIN	25	68.59772	28.39565	2.7	2.6	0.404	0.415	-0.028	n.s
	Kuutusoja	FKU	FIN	32	68.39260	28.05380	5.6	5.0	0.540	0.484	0.105	***
	Luttojoki	FFL	FIN	25	68.45248	28.39419	6.2	6.0	0.574	0.555	0.032	n.s
	Ruohojärvenoja	FFR	FIN	26	68.58249	28.01131	4.2	4.1	0.503	0.483	0.041	***
	Suomujoki	FFS	FIN	25	68.40924	28.22667	6.4	6.3	0.574	0.549	0.043	n.s

the Livojoki and Suomujoki populations of the Iijoki and the Tuloma drainage systems, respectively. The lowest genetic diversity was observed in the Hanhioja and Portinoja populations, also from the Iijoki and Tuloma drainages, respectively. The expected heterozygosity (H_E) per population was the lowest, 0.254, for Wolfsbach, Germany. Among the Finnish populations, the expected heterozygosity (H_E) per population varied between 0.380 for the River Sarriojoki (the River Kemijoki drainage), and 0.584, for Louttajohka (the River Tenojoki drainage). Observed heterozygosity (H_O) ranged between 0.245 (Wolfsbach, Germany) and 0.564 (Lohijoki, Iijoki drainage), while the lowest Finnish value, 0.360, was found in the River Sarriojoki. A summary of the microsatellite diversity indices is provided in Table 7.

Pair-wise F_{ST} values for pearl mussel populations spanned a wide range and 88% of differences in all pairwise comparisons were highly significant (P < 0.001) (Table 8). The strongest differentiation was detected between populations in central Europe (Zinnbach and Wolfsbach,) and Finnish populations (F_{ST} from 0.179 to 0.391). Among the Finnish populations, F_{ST} values ranged from 0.001 to 0.265. High values were detected between populations from different drainages (e.g. Lohijoki vs. Sarriojoki, F_{ST} = 0.217) as well as between populations of the same drainage (e.g. Hanhioja and Ruohojärvenoja, F_{ST} = 0.221) (Table 8).

The NJ phenogram illustrates the genetic relationships between populations based on Nei D_A (Nei et al. 1983) genetic distances (Fig. 5). Only a number of populations from one drainage system are clustered together (e.g. Luttojoki, Kuutusoja and Suomujoki from Tuloma drainage and Koutusjoki and Luomalanjoki from the River Torniojoki drainage). Even if geographical distance (waterway) is short between populations within drainage systems (e.g. Tuloma and Torniojoki), the populations are separated with long-branch lengths and statistically robust nodes from one another (e.g. Hanhioja to Suomujoki, Luttojoki, Kuutusoja, and Ruohojärvenoja from Tuloma drainage; Portinoja to Livojoki and Lohijoki from Iijoki drainage, Fig. 5).

When the population-specific mean values were used as the statistical unit, both the mean average number of alleles per locus (*A*) and the mean allelic richness per population (A_R) was higher in salmon rivers (n = 3) than in brown trout rivers (n = 12). The mean average number of alleles per locus (± standard error of mean) was 6.50 ± 0.21 and 4.86 ± 0.31 in salmon and brown trout rivers, respectively, the difference being statistically significant (Mann-Whitney U test, p = 0.004). The mean allelic richness per population was 6.30 ± 0.17 and 4.72 ± 0.30 (Mann-Whitney U test, p = 0.004), respectively. In the haplotype diversity index, such a difference was not found. Similarly, for mean expected heterozygosity and mean observed heterozygosity no statistically significant differences were observed. The mean expected heterozygosity (H_E) over populations was 0.56 ±0.01 and 0.51 ± 0.02 in salmon and brown trout rivers, respectively (ANOVA, $F_{1,15}$ = 1.894, p = 0.189). The mean observed heterozygosity (H_O), on the other hand, was 0.54 ± 0.12 and 0.49 ± 0.17 in salmon and brown trout rivers, respectively (ANOVA, $F_{1,15}$ = 2.046, p = 0.173). In those six brown trout rivers from which the estimated number of mussels (census) as well as microsatellite data were available, there was no statistically significant correlation between the mean number of alleles per locus (*A*) and *M. margaritifera* population size (Spearman correlation coefficient = 0.348, p = 0.499, n = 6).

The mitochondrial DNA and microsatellite results correlated with each other when analysed using population-specific values as a statistical unit (a total of 15 *M. margaritifera* populations with both mtDNA and microsatellite data). There was a statistically significant, positive correlation between the observed mtDNA haplotype richness (number of haplotypes per population, H) and the mean number of microsatellite alleles per locus (*A*) (Pearson correlation coefficient = 0.598, p = 0.019, n = 15). In addition, there was also a significant positive correlation between the expected mtDNA haplotype richness (H_R) and mean microsatellite allelic richness (A_R) over populations (Pearson correlation coefficient = 0.573, p = 0.026, n = 15). However, the correlation between the calculated diversity index of mtDNA haplotypes (h) and the expected microsatellite heterozygosity (H_E) was not statistically significant (Pearson correlation coefficient = 0.215, p = 0.441, n = 15).

Table 8 Pairwise estimates of F_{ST} values (based on microsatellite data) according to Weir and Cockerham (1984) (significant values ($P < 0.001$) in bold). For river codes see Fig 1.

Drainage		DWB	DZI	FLH	FHU	FLI	FPO	FSR	FSA	FSI	FON	FLO	FJU	FKO	FLU	FHA	FKU	FFL	FFR
Elbe	DZI	0.058																	
Iijoki	FLH	**0.317**	**0.209**																
	FHU	**0.334**	**0.253**	**0.127**															
	FLI	**0.304**	**0.197**	0.038	**0.143**														
	FPO	**0.391**	**0.276**	**0.157**	**0.198**	**0.134**													
Kemijoki	FSR	**0.371**	**0.302**	**0.217**	**0.133**	**0.191**	**0.180**												
	FSA	**0.277**	**0.194**	**0.059**	0.046	0.070	**0.156**	**0.133**											
	FSI	**0.365**	**0.288**	**0.146**	**0.132**	**0.151**	**0.181**	0.044	**0.112**										
	FON	**0.259**	**0.183**	**0.128**	**0.136**	**0.092**	**0.156**	**0.137**	**0.073**	**0.149**									
Teno	FLO	**0.302**	**0.199**	**0.056**	**0.132**	**0.035**	**0.105**	**0.184**	**0.087**	**0.147**	**0.123**								
Tornionjoki	FJU	**0.310**	**0.221**	**0.038**	**0.127**	0.038	**0.144**	**0.181**	**0.055**	**0.125**	**0.103**	**0.065**							
	FKO	**0.278**	**0.186**	**0.066**	**0.145**	0.010	**0.120**	**0.147**	**0.063**	**0.112**	**0.063**	**0.067**	**0.044**						
	FLU	**0.308**	**0.229**	**0.089**	**0.168**	**0.060**	**0.177**	**0.173**	**0.086**	**0.103**	**0.102**	**0.079**	**0.078**	0.040					
Tuloma	FHA	**0.372**	**0.261**	**0.168**	**0.166**	**0.102**	**0.170**	**0.185**	**0.138**	**0.195**	**0.144**	**0.121**	**0.173**	**0.117**	**0.182**				
	FKU	**0.306**	**0.201**	**0.061**	**0.126**	0.036	**0.111**	**0.179**	**0.057**	**0.158**	**0.095**	**0.029**	**0.075**	**0.052**	**0.089**	**0.101**			
	FFL	**0.295**	**0.186**	0.046	**0.135**	0.018	**0.094**	**0.190**	**0.064**	**0.153**	**0.079**	0.018	**0.052**	0.040	**0.071**	**0.098**	**0.074**		
	FFR	**0.323**	**0.229**	**0.125**	**0.205**	**0.080**	**0.208**	**0.265**	**0.137**	**0.230**	**0.169**	**0.078**	**0.107**	**0.105**	**0.124**	**0.221**	0.001	**0.060**	
	FFS	**0.289**	**0.179**	**0.056**	**0.132**	0.033	**0.127**	**0.208**	**0.065**	**0.170**	**0.098**	0.021	**0.065**	0.052	**0.077**	**0.121**	0.001	0.001	0.045

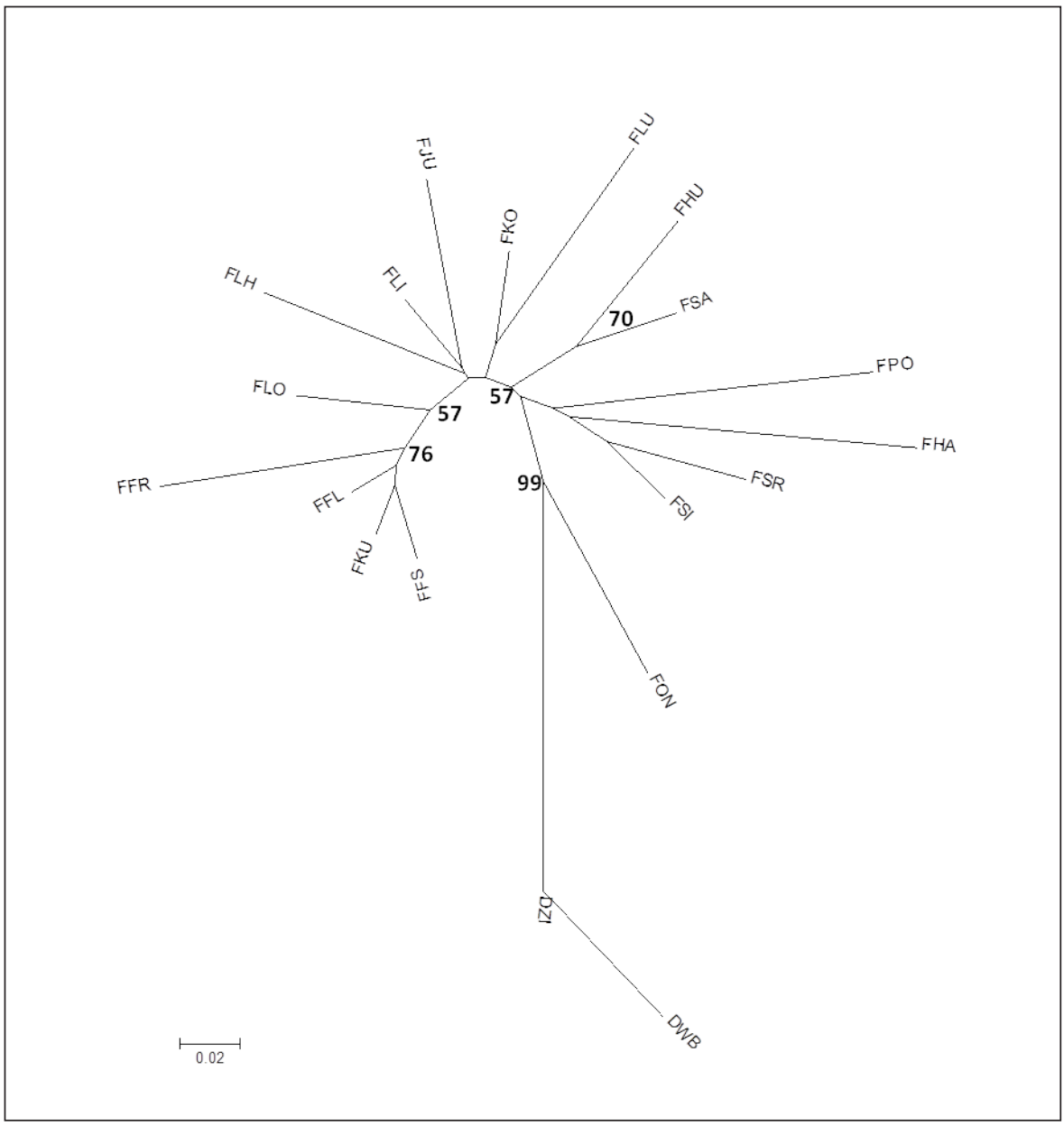

Figure 5. Neighbor-joining (NJ) phenogram based on Nei D_A (Nei et al. 1983) genetic distance for Finnish and German freshwater pearl mussel populations based on microsatellite data. Numbers indicate nodes with bootstrap support of more than 50% for 1000 replications. DWB=Wolfbach, DZI=Zinnbach, FLH=Lohijoki, Hukkajoki=FHU, Livojoki=FLI, Portinoja=FPO, Sarriojoki=FSR, Saukkojoki=FSA, Siikajoki=FSI, Onnasjoki=FON, Louttajohka=FLO, Juojoki=FJU, Koutusjoki=FKO, Luomalanjoki=FLU, Hanhioja=FHA, Kuutosoja=FKU, Luttojoki=FFL, Ruohojärvenoja=FFR, Suomujoki=FFS.

Discussion

Results of mtDNA and microsatellite data analysis were largely consistent with each other, in other words, results were congruent. Both F_{ST} values and NJ phenogram indicate a structured genetic differentiation pattern of pearl mussel populations, suggesting that different conservation units should be considered in the management of the species. Additionally, there was no correlation in the observed genetic population structure between populations within certain drainage system. These results match previous observations from central Europe and Sweden, where the link between geographic distance and genetic differentiation was also not evident (Geist & Kuehn 2005; Geist et al. 2010).

The successful conservation of the freshwater pearl mussel populations requires substantial financial resources, since it requires covering the entire freshwater pearl mussel life cycle, including proper management of host fish stocks and water catchment areas. In such a

case, knowledge of the genetic structure of the populations is essential for successful and effective conservation, because it allows allocating limited financial resources to so-called priority populations. Populations which are characterized by a high genetic diversity, private alleles (unique haplotypes) and high F_{ST} values can be considered as such priority populations, because they represent "nature's own gene banks".

Higher levels of genetic diversity e.g. haplotype richness, the number of alleles per locus, and allelic richness were found in salmon rivers as compared to brown trout rivers. Present results indicate that this may be due to the more isolated nature of mussel populations in the brown trout rivers. The highest pearl mussel numbers were observed in the salmon rivers Suomujoki (133,561 individuals) and Karpelva (700,000 individuals). In Luttojoki, pearl mussel stock on the Finnish side was estimated to be at least 150,000 individuals (Oulasvirta 2010b). Although the maximum observed mussel population size among brown trout rivers was also high, 131,478 individuals in Koutusjoki, the census estimates for salmon rivers seem usually to be higher. On the other hand, the brown trout rivers are mostly small headwater tributaries which may have isolated, resident brown trout as the host, limiting opportunities for migration, and gene exchange between mussel populations. In different drainage systems, high genetic diversities were observed in the Rivers Livojoki, Luttojoki, Koutusjoki, Skjellbekken, Karpelva, Siikajoki and Onnasjoki (of the Iijoki, Tuloma, Torniojoki, Pasvik, Karpelv and Kemijoki drainage basins, respectively). Conservation of these rivers and catchment areas should be a high priority. Large population size was connected to higher number of mtDNA haplotypes and higher expected haplotype richness, although the haplotype richness could be high even in small mussel populations. In addition, in the large sized pearl mussel populations, the number of microsatellite alleles per locus was also high. Thus, the present results indicate that larger *M. margaritifera* numbers in a given river favour maintenance of diverse haplotypes and genotypes. This means that although genetic richness can be substantially high even in small mussel populations, high mussel densities and large mussel stocks should be the target of conservation efforts for the sake of genetic diversity of *M. margaritifera*. Although there were some positive F_{IS} values (Table 7), which may indicate inbreeding, the majority of these values were still near to zero. For example, positive F_{IS} values and statistically significant Hardy–Weinberg probability tests of the Rivers Louttajohka (0.109) of the Teno drainage basin and Kuutusoja (0.105) of the Tuloma drainage basin may indicate heterozygote deficiency (inbreeding) in these populations. Also in the River Livojoki there was positive F_{IS} value (0.037) and the Hardy–Weinberg probability test was statistically significant. Because the River Livojoki pearl mussels have one of the highest genetic diversities in this study area, it would be important to support its natural glochidia reproduction.

Most of the populations in this study had relative high pairwise F_{ST} values, and in some populations there were also private alleles, indicating that there is a strong differentiation between those populations. Thus, no introductions should be carried out to increase genetic diversity or decrease inbreeding, because this could lead to outbreeding depression, which means a reduction in fitness by breakdown of the locally developed genetic adaptations (Templeton 1986).

The results of this study show that current population differentiation does not match with current drainage systems. This can be explained by historical changes in rivers, tributaries and drainage systems. Another likely explanation can be found in the colonization or the population history. As the ability of an adult freshwater pearl mussel to move independently is very limited, its colonization is completely dependent on the migration of host fish species (Bauer 1987). For this reason, it can be assumed that the colonization history of the freshwater pearl mussel is connected with the colonization history of its host fish species (brown trout and salmon). Also many anthropogenic factors, such as habitat alteration and destruction, pearl fishing and exploiting host fish stock, may have affected the genetic population structure. That is because these actions could lead to local extinctions, fragmented habitats and populations, and smaller population densities, which could lead to processes like genetic drift and inbreeding.

Annex E

Host fish and cultivation experiments

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1 Introduction

An important part of the life cycle of the freshwater pearl mussel, *Margaritifera margaritifera*, is the parasitic stage in the gills of its fish host. Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* are the fish hosts of European *M. margaritifera*, while in the North America the brook trout, *Salvelinus fontinalis*, has also been suggested as serving as the host (Smith 1976). In Europe, salmon and brown trout are usually thought to be equally suitable hosts for the pearl mussel. However, in Norway a number of rivers have been characterized as almost exclusively parasitizing either Atlantic salmon or brown trout, even when both hosts are present (Larsen and Karlsson 2012). If salmon- or trout-dependence were a wider phenomenon, and especially if it occurred in the current project area, it would have important consequences for the conservation of *M. margaritifera*. Many large northern rivers such as River Luttojoki, River Suomujoki, River Simojoki, River Iijoki, River Livojoki and River Karpelva are known to contain Atlantic salmon, or have been salmon rivers before. If pearl mussels in a (former) salmon river prefer salmon as their host, this would urge conservation or restoration of the migratory salmon populations in those rivers. It would also challenge the current management of, for example, the River Iijoki area salmon, as young salmon have not been stocked e.g. in the former salmon tributary River Livojoki for more than 50 years.

Local adaptation of the freshwater pearl mussel to the local host population has only rarely been studied. The results of Buddensiek (1995) indicated that pearl mussels may infect the local brown trout better than the non-local brown trout. Theoretically, in the long run, host-parasite coevolution should lead to local adaptation, i.e. adaptation of the parasite to the local host population. In practice, the incompatibility between host and parasite should increase with geographic distance. While the local host should be the best, the host population with greatest distance from the mussel should be the worst. If local adaptation occurred more commonly in *M. margaritifera*, it should be taken into account, for example, in possible restoration, replanting and cultivation projects for the freshwater pearl mussels.

The North American species, brook trout, was introduced to northern Finland in the 1950s and has been stocked in many small rivers there. Brook trout, an invasive species in Europe, has been suggested to be the fish host for *M. margaritifera* in North America (Smith 1976), but it is not very well known whether it is a suitable host for the European freshwater pearl mussel. Studies by Bauer (1987) and Jung et al. (2013) suggest that brook trout is an unsuitable host for *M. margaritifera*. However, the northern Finnish *S. fontinalis* may be of a different origin from the stocks in Central Europe, and may have been in contact with European *M. margaritifera* for a different time span. Therefore, it is important to study the local situation here in the north. If brook trout is not a suitable host, the spread of brook trout would pose a risk to the endangered *M. margaritifera*, as it may negatively affect the local pearl mussel host, brown trout (Korsu et al. 2007).

Thus, the main target of the project was to study the potential differences in the suitability of brown trout and Atlantic salmon, as well as

brook trout as a host fish for the freshwater pearl mussel. In addition, the aim was to investigate differences in the suitability between local (sympatric) and non-local (allopatric) populations of these species, in different rivers. These questions were studied by field (cage) and laboratory (tank) infection experiments, with pearl mussels and host fish from the River Iijoki, River Simojoki and River Luttojoki catchment areas.

The process associated with suitability of fish as host, namely the possible acquired immunity of a host fish against pearl mussel glochidium larvae, was also studied in a laboratory experiment. It has been shown that fish might develop immunity against the pearl mussel infection (Bauer & Vogel 1987, Treasurer et al. 2006) after their first exposure to glochidia. This should, however, also be verified in the present northern latitudes.

Many of the freshwater pearl mussel populations are threatened or even extinct, and there is a need for artificial cultivation methods. Indeed, such projects have recently been started in Europe. Thus, the target of the present project was also to conduct preliminary studies on laboratory rearing of *M. margaritifera* glochidia and juveniles, so as to test infection of juvenile salmonids on fish farms with pearl mussel glochidia, and to examine the results of a previous planting experiment of lab-reared *M. margaritifera* juveniles in the Iijoki area.

The following terminology and names of the species and strains will be used in this report:

Abundance of infection..... means the mean number of glochidia per fish

Atlantic salmon..... also: salmon, *Salmo salar*, *S. salar*

Brook trout ..... also: *Salvelinus fontinalis*, *S. fontinalis*

Brown trout ..... also: *Salmo trutta*, *S. trutta*

Freshwater pearl mussel..... also: pearl mussel, mussel, *Margaritifera*, *M. margaritifera*, FWPM

Local..... means 'from the same location (sympatric)'

Non-local..... means 'from a different location (allopatric)'

Prevalence of infection ..... means proportion of fish infected (%); also: infection rate, infestation rate, encystment rate

Resident fish..... means fish that will mainly stay in the river throughout their life, or short migrate to nearby lakes

River Iijoki brown trout ..... also: sea-migrating River Iijoki brown trout, means brown trout that used to spawn in River Iijoki and migrate to the sea for feeding, now maintained in a hatchery; other strains of brown trout in River Iijoki catchment were completely resident, or perhaps made shorter migrations to nearby lakes and they are later called resident brown trout

River Iijoki salmon..... means sea-migrating River Iijoki Atlantic salmon that used to spawn in River Iijoki and migrate to the sea for feeding, now maintained in a hatchery

Sea-migrating..... also: anadromous; migrates to the sea for feeding, returns to the river for spawning; the opposite of resident

Stock..... means a farmed strain

Strain ..... also stock; genetically, behaviourally and/or geographically distinct groups within a species

2 Study areas

The River Iijoki, River Luttojoki and River Simojoki basins were used for caging experiments (Fig. 1). The experiments in the Iijoki area were carried out 2011–2013, and in the Luttojoki area in 2012; caging in River Simojoki was included to the study in 2013

The study rivers are listed in Table 1. In the River Iijoki catchment, the large tributary, River Livojoki, was once a spawning area of anadromous (sea-migrating) Atlantic salmon and River Iijoki brown trout before River Iijoki was dammed in the 1960s. All the other rivers or streams in the Iijoki area used in the caging experiments, as well as River Jukuanoja, from which the mussel larvae were obtained in a laboratory experiment in 2012, are thought to been populated only by brown trout. It is not exactly known which of the tributaries were used by sea-migrating River Iijoki brown trout, and which by the more resident brown trout, but at least River Ala-Haapuanoja and River Porraslammenoja, maybe also River Koivuoja and River Jukuanoja, should have been sea-migrating River Iijoki brown trout rivers. There was also a brook trout population in some of the study rivers, for example in River Porraslammenoja, in River Iijoki catchment.

River Simojoki has a natural, reproducing Atlantic salmon population. Atlantic salmon also used to ascend to River Luttojoki in the River Tuloma catchment, but salmon migration was stopped in the 1960s by a hydroelectric power plant on the Russian side of the river.

3. Methods

3.1 Caging experiments

The caging experiments in the River Iijoki catchment area in 2011–2013 were carried out in a total of 7 rivers: Portinjoki, Lohijoki, Koivuoja and Porraslammenoja in the municipality of

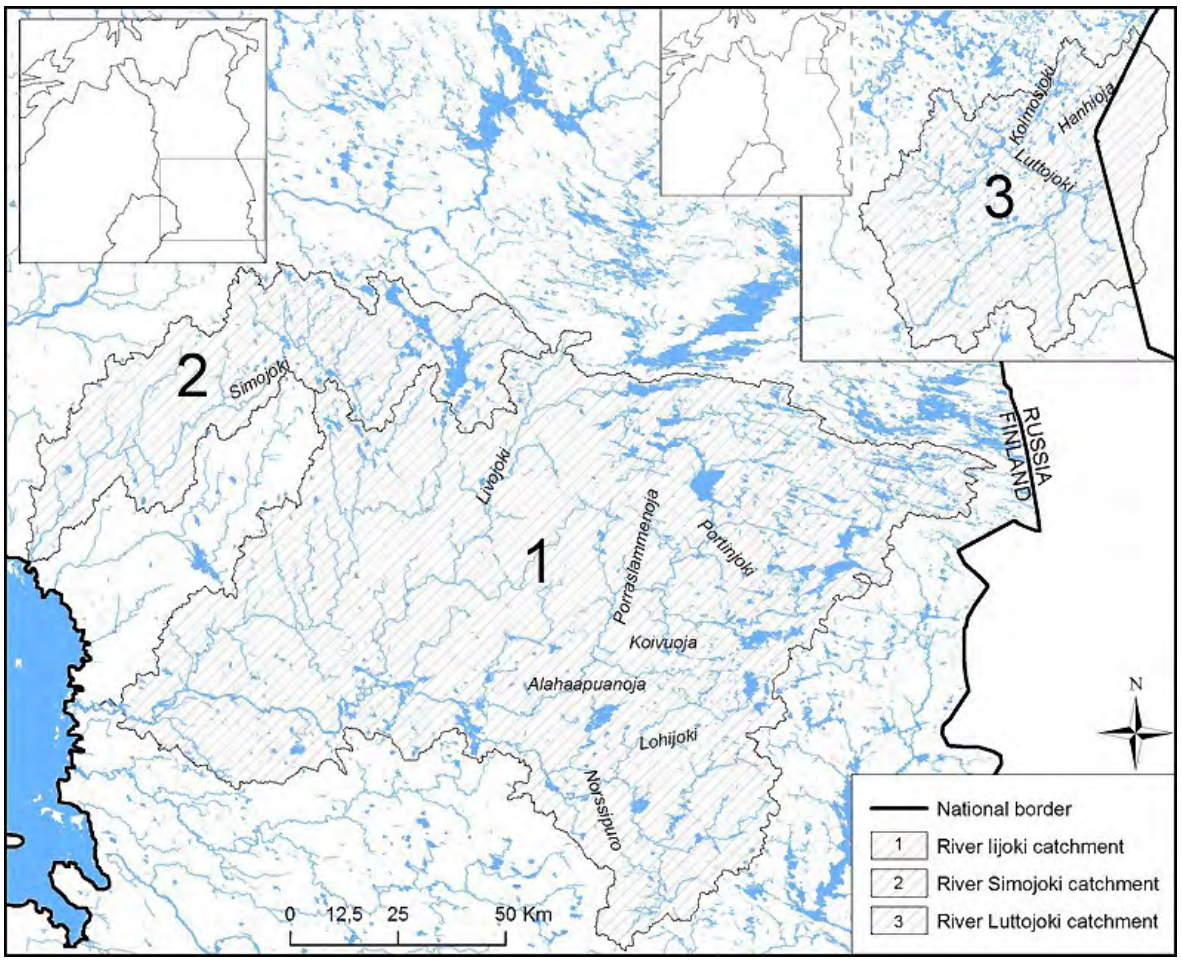

Figure 1. Catchments of River Iijoki (1), River Simojoki (2) and River Luttojoki (3) used in 2011–2013 field experiments to study the suitability of different salmonid fish species and strains as the host of *M. margaritifera*. © Metsähallitus 2015, © National Land Survey of Finland 1/MML/15.

Table 1. Catchments, rivers, salmonid species, stocks and populations, caging dates and numbers of cages used in 2011–2013 field experiments to study suitability of different fish as the host of *M. margaritifera*, including a rough estimate of mussel population size.

Year	Catchment	River	Number of mussels	Date	Salmon			Brown trout												Brook trout	Number of cages
					Ii	Tornio	Simo	Ii	Rauta	Kitka	Lohi	Portin	Porrasl.	Alah.	Norssi	Laiva	Lutto	Kolmos	Hanhi		
2011	Iijoki	Lohijoki	25,000	05.09.–21.09.	●			●			●	●									9
		Koivuoja	2,000	07.09.–27.09.	●			●			●										7
		Portinjoki	5,000	06.09.–20.09.	●			●			●	●									9
		Livojoki	3,500	08.09.–11.10.	●			●	●	●											9
2012	Iijoki	Porraslammenoja	1,000	27.08.–18.10.	●			●	●				●							●	10
		Alahaapuanoja	1,500	28.08.–17.10.	●			●	●					●							8
		Norssipuro	20,200	29.08.–16.10.	●			●	●						●						8
		Livojoki	8,500	30.08.–18.10.	●			●	●	●						●					10
	Luttojoki	Hanhioja	15,700	23.08.–04.10.													●	●	●		6
		Kolmosjoki	1,000	22.08.–04.10.													●	●	●		6
2013	Iijoki	Livojoki	3,500	20.08.–17.10.	●	●	●	●													12
	Simojoki	Simojoki	500	21.08.–14.10.	●	●	●	●													12

Farmed fish | Natural fish

Taivalkoski, and Livojoki, Alahaapuanoja and Norssipuro in the municipality of Pudasjärvi (Table 1). In the River Luttojoki area, fish were caged in the tributaries Hanhioja and Kolmosjoki in 2012. The caging experiment also included River Simojoki in 2013. Caging dates and fish origins in each river are listed in Table 1.

In cases when wild fish were used, the work was begun by catching juvenile brown trout by electrofishing from the rivers where needed, with a target to catch same sized 0+ year class fish to make sure that none of the fish were exposed to pearl mussel larvae before the experiment. In 2011 and 2012, after they were caught, the fish were maintained for a few days in two 3 m^3 outdoor tanks before they were moved to the cages, but this procedure was not applied in 2013.

In 2011, fish from every population were put into two replicate cages (only one fish species/stock/origin per cage) with 15 to 25 individuals per cage. In 2011, there was also a monitoring cage (containing River Iijoki brown trout), which was used to monitor the appearance of pearl mussel glochidia in fish in order to find the optimal end time for the caging. In 2011, some of the caged fish were kept for a longer period (see below), but in 2012–13 all fish from each cage were killed and examined at the same time, as late as possible, about 1.5 months after the caging was started. Otherwise, the methods were the same in 2012–13 as in 2011, except that three replicate cages per population were used in 2013. In addition, in the River Iijoki catchment in 2012, the River Iijoki Atlantic salmon and River Iijoki brown trout were put into same cages in total of four replicates. Fish were obtained from the Finnish Game and Fisheries Research Institute (FGFRI) Taivalkoski fish farm and Kalankasvatus Vääräniemi's Oudonjoki fish farm, both located in the River Iijoki drainage.

In the River Livojoki cage experiment in 2012, the resident brown trout used were caught from nearby River Laivajoki, because the main channel of River Livojoki does not contain anymore its original brown trout strain like River Laivajoki. Farmed brown trout of the River Kitkajoki and Rautalampi strain (the latter also sometimes called Lake Konnevesi stock) were chosen for caging experiments because both strains are widely used in restocking in Finland. These farmed fish, as well as brook trout and the sea-migrating River Iijoki brown trout and River Iijoki salmon, were obtained from FGFRI Taivalkoski.

In the River Luttojoki area in 2012, the rivers/streams for caging were selected according to their different history of salmonid fish. River Hanhioja is a small stream with a resident brown trout population. River Kolmosjoki is a larger tributary which now includes a resident brown trout population. The fish from the upper part of River Luttojoki were caught from the area above the freshwater pearl mussel distribution range.

The order of the cages in river was otherwise randomized, but the replicate cages containing fish from the same population were not placed next to each other (Figure 2).

Figure 2. Fish cages in a tributary of River Iijoki in the 2011 field experiment to study the suitability of different fish species, stocks and populations as the host of freshwater pearl mussels. Photo Metsähallitus.

After caging, fish were killed, stored on ice and transported to the laboratory. In the laboratory, the fish were measured for length and weight. After that, the gill arches were cut off, and *M. margaritifera* glochidium larvae were counted microscopically by pressing the gills between two large glass plates (Fig. 3). A subsample of 10–15 glochidia from each fish was also measured for length with a scaled microscope. In 2011, however, caged fish were transported to the Konnevesi research station and kept individually in 15 litre plastic flow-through thanks in order to follow the development of glochidia for a longer period. Thus, in the 2011 experiment the fish were examined on several occasions from the autumn of 2011 to the spring of 2012.

In River Norssipuro 2012, the infection by glochidia failed, possibly due to starting caging too late. Infection of River Laivajoki brown trout caged in River Livojoki 2012 also failed, as the fish had already become infected in their river of origin. In addition, one cage was damaged, and the fish lost, in River Porraslammenoja in 2012.

3.2 Laboratory experiments

In 2011, individuals of (a) brook trout, (b) River Iijoki brown trout and (c) River Iijoki Atlantic salmon, and brown trout of three different strains – (d) Rautalampi, (e) River Isojoki and (f) River Luutajoki – all obtained from Finnish game and Fisheries research Institute fish farms, were infected with pearl mussel glochidia originating from River Koivuoja (a small brown trout tributary of River Iijoki). There were two replicate tanks per fish strain, except for brook trout and River Luutajoki brown trout which were in one tank per strain only. The number of fish per tank was about 30. River Isojoki, which is in western Finland and flows directly into the Baltic Sea, has its own *M. margaritifera* population, which, however, is at present almost extinct. River Luutajoki is also situated in western Finland. It belongs to the River Kokemäenjoki catchment in which freshwater pearl mussel is known from two rivers, but not from River Luutajoki. The collection of glochidia was carried out following Young & Williams (1984a, b) and Bauer (1987). *Margaritifera* were either directly put into a bucket filled with river water where they shed the glochidia, or they were first kept on shore for 15–30 minutes before placing them in buckets, to trigger the glochidia release. The glochidium suspension was then transported on ice to the laboratory of Konnevesi research station. Two litres of glochidium suspension, approximately 200,000 glochidia, were added to each experimental tank (water volume 163 l). During the infection, water flow in the tanks

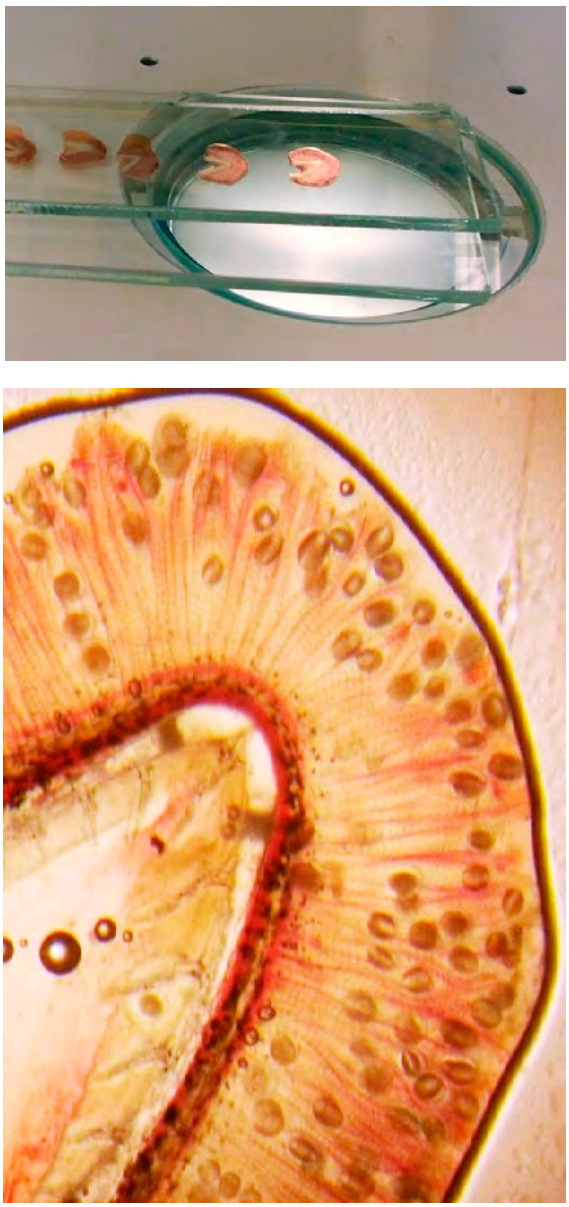

Figure 3. Gill arches of fish pressed between two glass plates in order to count the number and measure the size of *M. margaritifera* glochidia (darker round or oval structures in the lower picture) microscopically. Photos Jouni Salonen.

was stopped, the water level was lowered to 70 l, and external oxygenation was provided. The following winter, the fish were examined as after the caging experiments.

In 2012 the glochidia were collected from River Jukuanoja, a small brown trout tributary of River Iijoki, presumably a former sea-migrating brown trout river. The fish – (a) brook trout, (b) River Iijoki brown trout and (c) River Iijoki salmon, 100 individuals of each, all obtained from Finnish Game and Fisheries Research Institute Taivalkoski fish farm – were put into two replicate tanks, with 50 individuals of each species per tank. Infection with pearl mussel glochidia was performed as in 2011, the number of FWPM glochidia released into each tank was roughly 290,000. In 2012 the aim, in addition to host specificity, was also to study the possible acquired immunity of fish against glochidia infection. For this purpose, 50 individuals of 0+ River Iijoki Atlantic salmon was infected with a high dose (880,000 glochidia per tank) of River Jukuanoja glochidia, one third with a low dose (170,000 glochidia per tank) and the rest were kept as an uninfected control group.

In 2013 the glochidia were collected from the main channel of River Luttojoki. These larvae were used both in the immunization experiment (in which fish were challenged a second time for glochidia exposure) and in the host fish experiment (50 individuals of (a) River Iijoki salmon, (b) River Iijoki brown trout and (c) Rautalampi strain brown trout in four replicate tanks). The glochidium dose was about 600,000 glochidia per tank. In the host fish experiment, the Rautalampi fish were marked with an adipose fin cut. During the fin cut, all the fish were anaesthetized with MS-222. All the fish in the species comparison were 0+ year class and obtained from a fish farm. Salmon in the immunization experiment were aged 1+ year in the second year of the 2-year experiment.

In 2011, the size (and age) differences between fish species and strains were unavoidable. In 2012, predation of brook trout on other fish caused a decrease in the fish in the final material. In 2013, the remarkable loss of the salmon, and unsuccessful marking of Rautalampi brown trout caused problems.

3.3 Data analysis

3.3.1 Caging experiments

In 2011 in the River Iijoki catchment, due to the size, age and origin differences of the fish, only fish with a length less than 85 mm were included in the statistical analyses. This was done to ensure that every fish in the analyses was aged 0+ years, which means that their gill areas were comparable to each other at the time of infection. In addition, this way, despite the different origins, it was ensured that none of the fish was exposed to pearl mussel glochidium larvae previously, to avoid possible acquired immunity.

Comparisons of the numbers of larvae in fish were mostly done with ANOVA or Kruskal-Wallis's and Mann-Whitney's tests using IBM SPSS Statistics program. Infection prevalences between different fish species or populations were compared, when needed, with χ^2-test. The Bonferroni correction was used in case of multiple analyses on the same fish group. In each analysis, the data from replicate cages within the same population were combined before the statistical tests and figures given in this report.

The statistical methods used in the analyses of 2012 caging experiments in the River Iijoki catchment were the same as in the previous year. In 2012 all fish were 0+ year of age. This time we also inspected all the fish at the same time (1.5 months after the assumed infection) which enables analysis of the development of the larvae in different hosts (glochidium length increment). This was done with ANOVA, Kruskal-Wallis or Mann-Whitney as well.

In 2012 in the River Luttojoki field experiments, after the caging at River Hanhioja and River Kolmosjoki, a size difference of fish from different populations was observed. The local brown trout from River Hanhioja were the smallest while those from the upper part of the main channel of River Luttojoki were the largest. Therefore, to compare these populations, the number of larvae in fish had to be standardized with the length of the fish, so that the number of glochidia was divided by the square of fish length (and this finally multiplied by 1,000).

In caging experiments in River Iijoki and River Simojoki in 2013, all the fish were 0+ year class. Statistical analyses were performed as in previous experiments.

3.3.2 Laboratory experiments

In 2011, 2012 and 2013 laboratory experiments, the data were analysed with ANOVA, Kruskal-Wallis, Mann-Whitney and χ^2 tests. In 2013, the data from different trout strains were combined in each tank because of marking problems, and Atlantic salmon from the immunization experiment was used as a comparison group in the host species experiment, although they were one year older and located in different tanks.

4. Results

4.1 Caging experiments in River Iijoki catchment 2011

4.1.1 River Livojoki, a (former) salmon river

The number of glochidia in fish caged in River Livojoki was very low (on average less than 10 glochidia per fish, Fig. 4) in 2011. Still, the anadromous River Iijoki salmon was easily indicated as being the best host for the Livojoki pearl mussel population. All salmon became infected, while infection prevalence in the three different trout populations was only 11 to 44% (Figure 1; χ^2-test, $p<0.001$ in each case). Also the mean number of glochidia was significantly higher in River Iijoki salmon than in anadromous River Iijoki trout or River Kitkajoki trout (Mann-Whitney test, $p<0.001$ in both cases), the difference from Rautalampi trout being statistically only suggestive.

Within the brown trout populations, the only difference was found between River Iijoki and Rautalampi trout, the latter carrying more glochidia (Mann-Whitney test, $p=0.002$) (Fig. 4).

4.1.2 River Koivuoja, (presumably a former sea-migrating) brown trout tributary

In the River Koivuoja experiment, most of the fish were infected, the total mean being more than 100 glochidia per fish (Fig. 5). River Iijoki Atlantic salmon had a higher encystment rate (100% prevalence) and contained slightly more glochidia than the River Iijoki sea-migrating brown trout, but the difference was not statistically significant.

4.1.3 River Lohijoki, a brown trout tributary

Fish were heavily infected with glochidia in the River Lohijoki cages, the prevalence of infection being 100% in all fish groups, and the number of larvae exceeded 400 glochidia per fish in every host (Fig. 6). However, the differences between fish species and strains were not statistically significant.

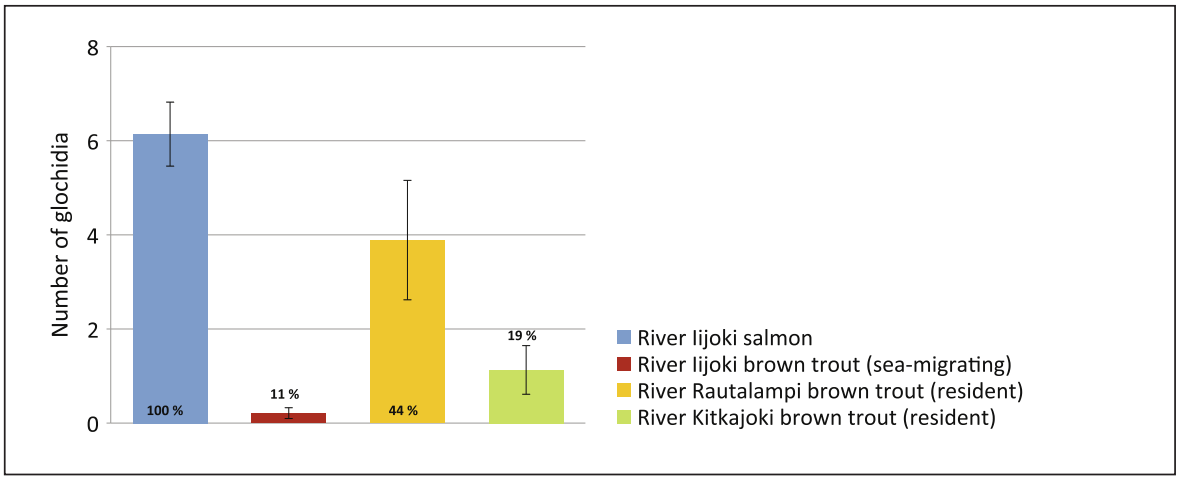

Figure 4. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish and infection prevalence (%) in different salmonid species and strains caged in River Livojoki in 2011. The infection rate in River Iijoki salmon was statistically significantly higher, in terms of mean glochidia abundance and prevalence of infection, than in any of the trout stocks. Fish length and age were the same in all fish groups.

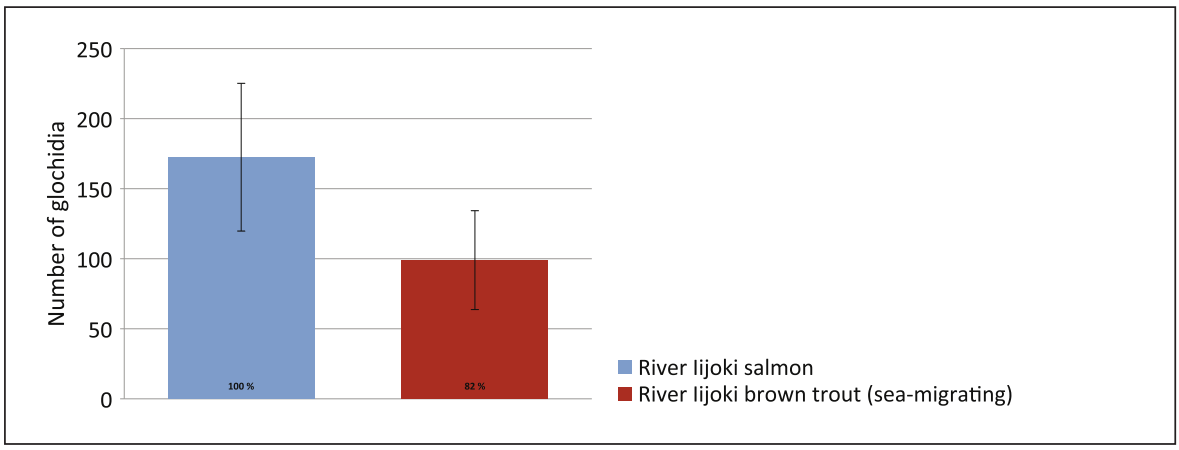

Figure 5. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish and infection prevalence (%) in River Iijoki salmon and trout caged in River Koivuoja in 2011. The difference between hosts in mean abundance of glochidia, or prevalence of infection, was statistically not significant. Fish length was the same in both fish groups.

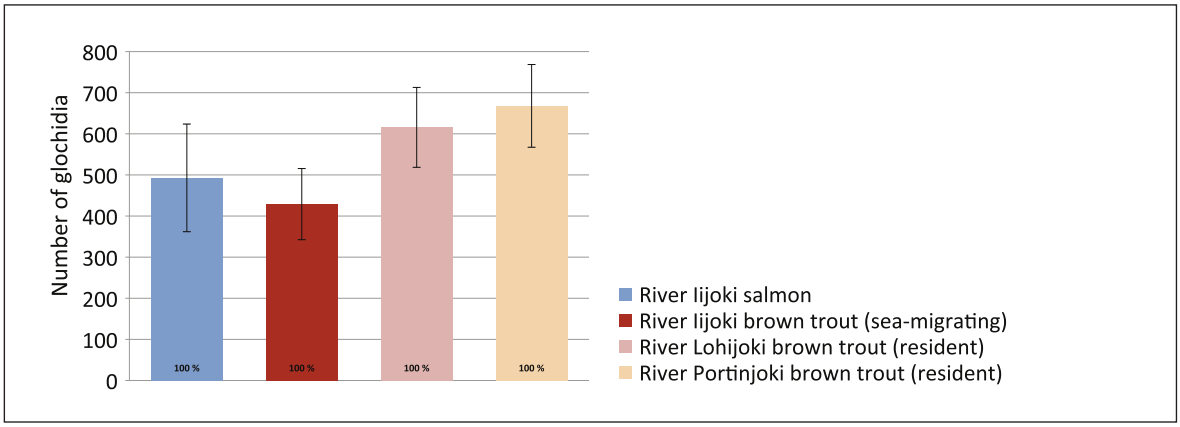

Figure 6. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish and infection prevalence (%) in different salmonid species and strains caged in River Lohijoki in 2011. The differences between hosts were not significant. Fish length was the same in all fish groups.

4.1.4 River Portinjoki, a brown trout tributary

Caging experiments in River Portinjoki resulted in very high mean numbers of glochidia (Fig. 7). The record was 2,962 glochidia in one of the resident River Portinjoki brown trout. The encystment rate was 100% in all fish groups, and there were no statistical differences between different brown trout populations in the mean number of glochidia per fish. However, the River Iijoki Atlantic salmon was a significantly worse host (Mann-Whitney test, $p<0.007$) than the sea-migrating River Iijoki brown trout or resident River Lohijoki brown trout (Fig. 7). However, the difference between River Iijoki Atlantic salmon and local River Portinjoki brown trout was not significant despite the large numerical difference – this was mainly due to the low number (n = 8) and large variation among River Portinjoki brown trout

4.2 Caging experiments in River Iijoki catchment 2012

4.2.1 River Livojoki, a (former) salmon river

Caging experiments in River Livojoki 2012 gave very similar results as in 2011 – almost every salmon (94%) became infected, while the infection prevalence was much lower in brown trout populations (31 to 70%) (Fig. 8). There was no difference in the mean number of encysted glochidia between River Iijoki Atlantic salmon and Rautalampi brown trout, but both of these carried statistically significantly more glochidia than River Kitkajoki or River Iijoki brown trout

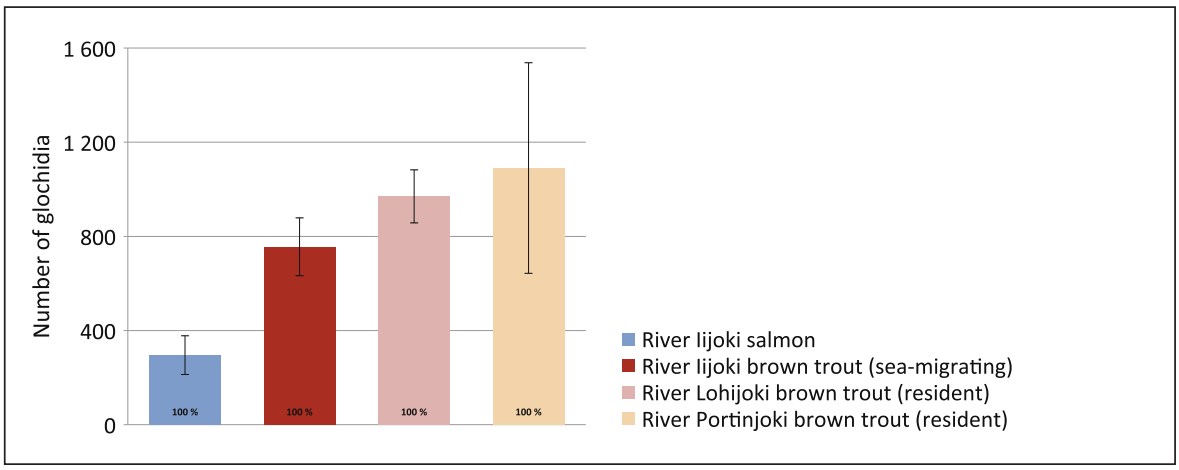

Figure 7. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish and infection prevalence (%) in different salmonid species and strains caged in River Portinjoki in 2011. In general, mean glochidia abundance was statistically significantly lower in salmon as compared to the trout stocks. Fish length was the same in all fish groups.

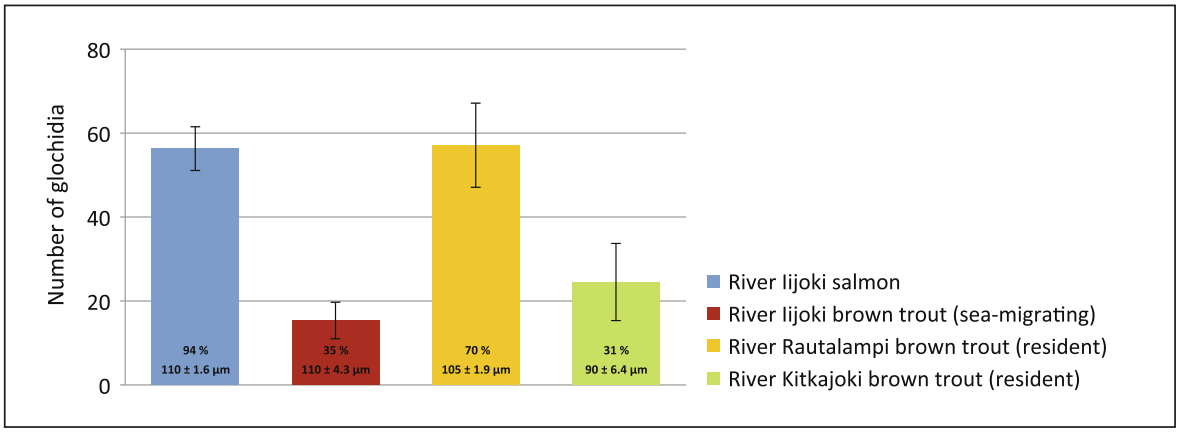

Figure 8. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish, infection prevalence (%) and mean length (± S.E.) of larvae in different salmonid species and strains caged in River Livojoki in 2012. Mean abundance and prevalence of infection was significantly higher in Iijoki salmon and Rautalampi trout than in the other fish hosts. River Iijoki salmon had a statistically marginally higher prevalence of infection than Rautalampi trout. There were no differences in the average length of glochidia between host fish strains. Fish length was the same in all fish groups.

(Mann-Whitney test, $p<0.003$) (Fig. 8). In addition to this, the encystment rate was statistically marginally higher in River Iijoki salmon than in Rautalampi brown trout. Even though the glochidia in River Iijoki salmon and brown trout seemed to be the largest (Fig. 8), no statistical differences in the size of larvae between different populations or species were found.

4.2.2 River Porraslammenoja, a (possible former sea-migrating) brown trout tributary

A heavy exposure to *Margaritifera* glochidia took place in River Porraslammenoja (Fig. 9), the maximum number of glochidia being 1,574 individuals in one Rautalampi brown trout. All the brown trout were infected, while the infestation rate in River Iijoki Atlantic salmon was 77%, which was significantly lower compared to River Iijoki brown trout or Rautalampi brown trout (χ^2-test, $p<0.001$ in both cases). The difference between River Iijoki salmon and the local River Porraslammenoja brown trout was also marginally significant, with a lower infection rate in salmon. Only 22% of the brook trout were infected, which was significantly lower compared to any other fish (χ^2-test, $p<0.001$ in each case). The mean number of glochidia was highest in Rautalampi brown trout, over 700 larvae per fish, which was statistically significantly higher value than in any other fish strain (Fig. 9). In River Iijoki Atlantic salmon, the number of glochidia was also significantly

lower than in Rautalampi brown trout, River Iijoki brown trout or River Porraslammenoja brown trout. The mean abundance of glochidia was lowest in brook trout (Mann-Whitney test, $p<0.003$ in all comparisons). The size of larvae ranged between 108 µm (brook trout) and 121 µm (brown trout Rautalampi), but statistically significant differences were not found.

4.2.3 River Ala-Haapuanoja, a (possible former sea-migrating) brown trout tributary

In River Ala-Haapuanoja, all brown trout strains were 100% infected with *Margaritifera* glochidia, whereas the infection prevalence among River Iijoki Atlantic salmon was 94% (Fig. 10). These prevalence differences were statistically only marginally significant, though. On the other hand, the mean number of glochidia was statistically significantly higher (Mann-Whitney test, $p<0.001$) in all brown trout strains than in River Iijoki salmon. There was also a significant difference between Rautalampi and River Iijoki brown trout (Mann-Whitney test, $p=0.007$). Finally, the local, resident River Ala-Haapuanoja brown trout carried more glochidia (over 230 individuals per fish) than any other fish (Fig. 10). Statistical differences in the size of glochidium larvae were not found between different fish strains or species.

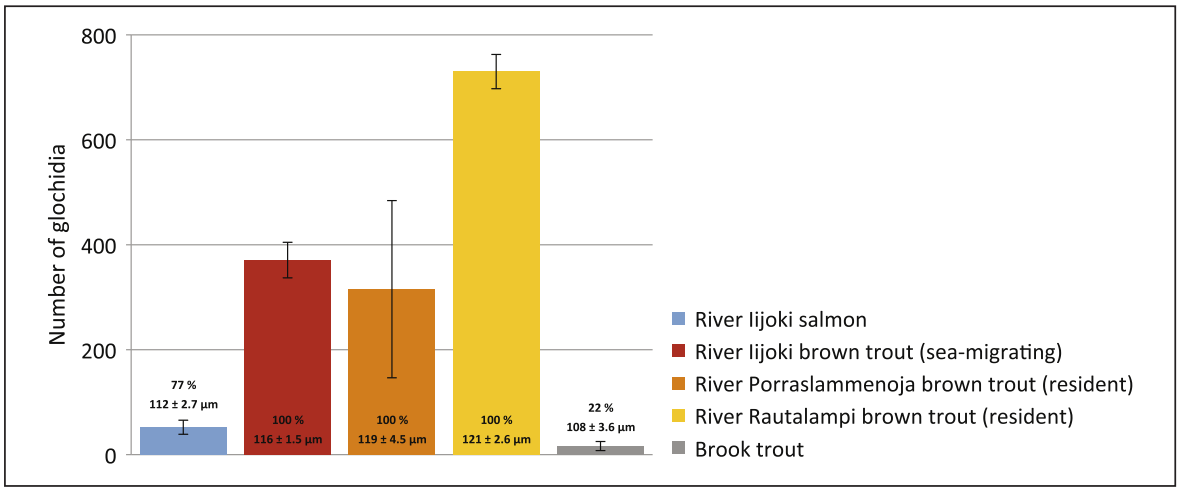

Figure 9. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish, infection prevalence (%) and mean length (± S.E.) of glochidia in different salmonid species and strains caged in River Porraslammenoja in 2012. As the diagram shows the lowest infection rate was in brook trout and second lowest in River Iijoki salmon, while the brown trout strains were the best hosts. Differences in glochidium size between the fish groups were not significant. Fish length was the same in all fish groups.

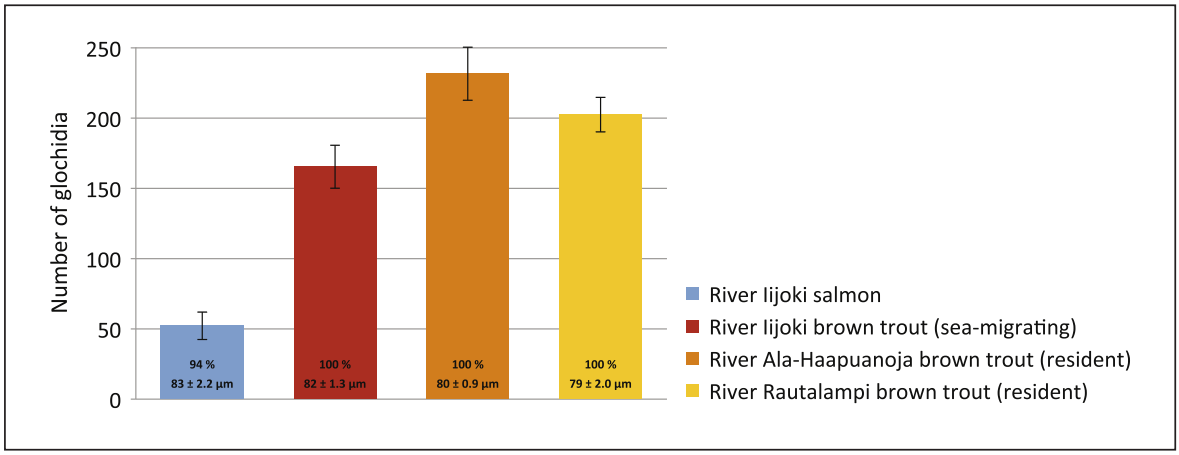

Figure 10. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish, infection prevalence (%) and mean length (± S.E.) of glochidia in different salmonid species and strains caged in River Ala-Haapuanoja in 2012. As regards the mean abundance of glochidia, the local River Ala-Haapuanoja brown trout was the best host, while River Iijoki salmon was the worst host for *Margaritifera*. Fish length was the same in all fish groups.

4.3 Caging experiments in River Luttojoki catchment 2012

4.3.1 River Hanhioja, a brown trout tributary

In Hanhioja, the infection rate was 100% in all brown trout strains. The local, resident River Hanhioja brown trout was infected with significantly (ANOVA, $p<0.015$) higher numbers of glochidia than the other brown trout strains (Fig. 11). Moreover, glochidia in the local, resident River Hanhioja brown trout were the biggest compared to other populations, while glochidia in River Kolmosjoki brown trout were the smallest ($p<0.001$) (Fig. 11).

4.3.2 River Kolmosjoki, presumably a brown trout tributary

As in River Hanhioja, every caged fish was parasitized with pearl mussel glochidium larvae in River Kolmosjoki. The local, resident Kolmosjoki brown trout carried statistically significantly (ANOVA, $p=0.014$) more larvae than the non-local Hanhioja trout (Fig. 12). Brown trout from the upper part of River Luttojoki was classified as the second best host, with a marginally significant difference (ANOVA, $p=0.058$) to River Kolmosjoki population. There were no statistical differences in the glochidium size between the fish groups.

4.4 Results of caging experiments in salmon rivers 2013

4.4.1 River Livojoki

The local River Iijoki Atlantic salmon was observed to be infested with the highest number of glochidia when caged in River Livojoki in the River Iijoki catchment (Fig. 13), one fish having up to 50 larvae in its gills. However, there were no statistical differences between the different Atlantic salmon populations in the mean number of glochidia, although the difference between the local, sympatric River Iijoki salmon and the allopatric River Tornionjoki salmon was marginally significant. Infection prevalence was 100% in every salmon cage, while in the Iijoki brown trout cages only 20–45 % of fish were infected (statistically significant difference; χ^2, $p<0.001$). The mean number of glochidia in River Iijoki brown trout was approximately 15 larvae per fish, which is significantly (Mann-Whitney test, $p<0.001$) lower than in any of the salmon populations (Fig. 13). The mean length of glochidia was also significantly lower in River Iijoki brown trout (Mann-Whitney test, $p<0.001$) than in any salmon population, indicating a lower growth rate of glochidia in trout (Fig. 13). However, there was also a difference in the size of larvae between Simojoki and Tornionjoki salmon, larvae in Simojoki salmon being statistically bigger (Mann-Whitney test, $p=0.003$).

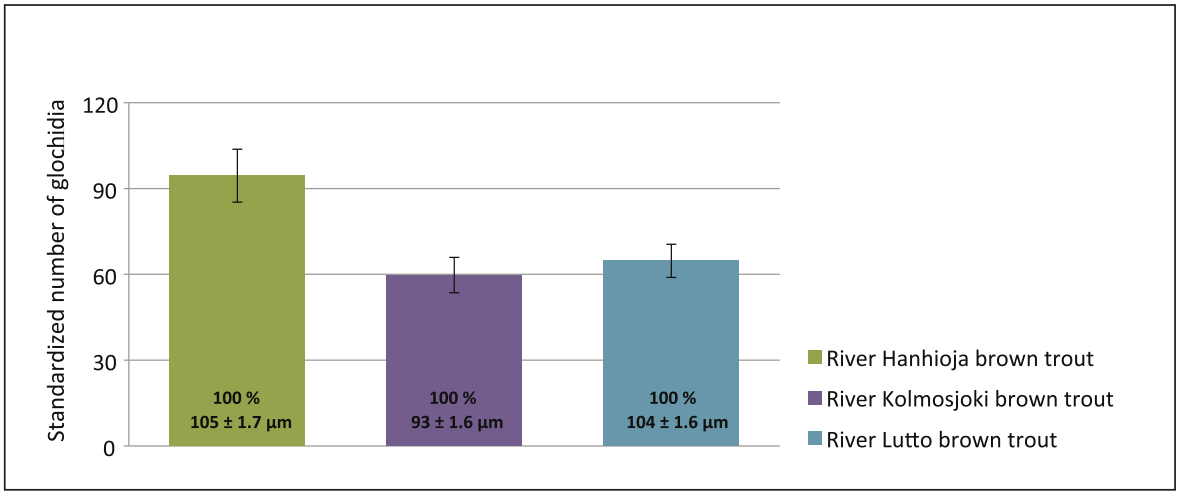

Figure 11. Mean fish-length-standardized number (± S.E.) of freshwater pearl mussel glochidia per fish, infection prevalence (%) and mean length (± S.E.) of glochidia in different brown and strains caged in River Hanhioja, River Luttojoki catchment, in 2012. The number of glochidia and glochidium growth rate (size) were highest in the local, resident River Hanhioja brown trout. Due to size differences between fish strains, the numbers of glochidia were standardized according to host fish length.

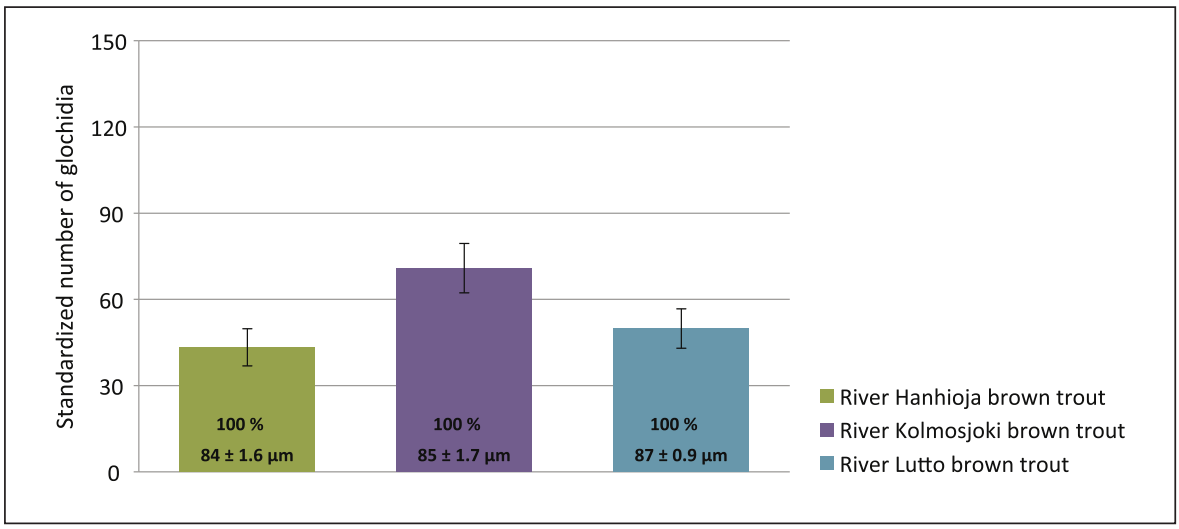

Figure 12. Mean fish-length-standardized number (± S.E.) of freshwater pearl mussel glochidia per fish, infection prevalence (%) and mean length (± S.E.) of glochidia in different brown trout strains caged in River Kolmosjoki, River Luttojoki catchment, in 2012. According to statistical tests, the number of glochidia was highest in the local, resident River Kolmosjoki brown trout. Due to size differences between fish strains, the numbers of glochidia were standardized according to host fish length.

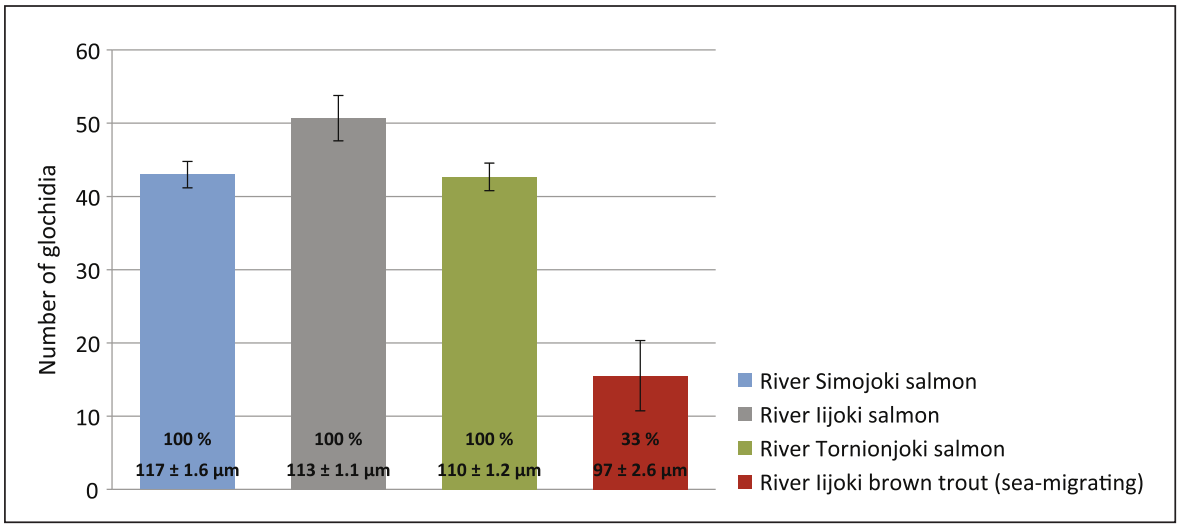

Figure 13. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish, infection prevalence (%) and mean length (± S.E.) of glochidia in different salmonid species and strains caged in River Livojoki, 2013. The worst host in all terms was the sea-migrating River Iijoki brown trout. Individuals of different salmonid species and strains were of the same size by their length.

4.4.2 River Simojoki

In general, the infestation rate and mean number of glochidia were clearly lower in River Simojoki caging than in River Livojoki caging. Otherwise the result was the same; all salmon strains were equally good as a host fish and significantly better in terms of number of glochidia (Mann-Whitney test, $p<0.002$) and infection rate (χ^2-test, $p<0.001$) than the sea-migrating River Iijoki brown trout (Fig. 14). No statistical differences in the size of glochidia between fish were found.

4.5 Laboratory experiments

4.5.1 Laboratory experiments with different host fish in 2011 and 2012

(a) Sea-migrating River Iijoki brown trout, (b) River Iijoki Atlantic salmon, (c) brook trout and (c) brown trout from four different strains were infected artificially in a laboratory experiment in 2011 with the pearl mussel larvae originating from River Koivuoja (presumably a former sea-migrating brown trout tributary in River Iijoki catchment).The non-local brown trout from River

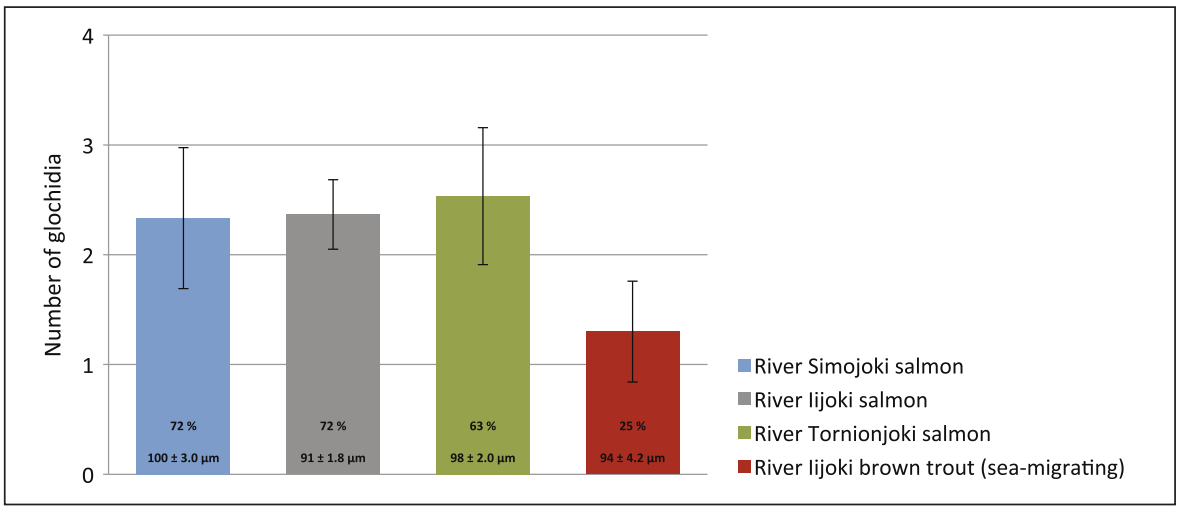

Figure 14. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish, infection prevalence (%), and mean length (± S.E.) of glochidia in different salmonid species and strains caged in River Simojoki, in 2013. The poorest host in terms of infection prevalence and abundance of infection was the River Iijoki brown trout. Individuals of different salmonid species and strains were of the same size by their length.

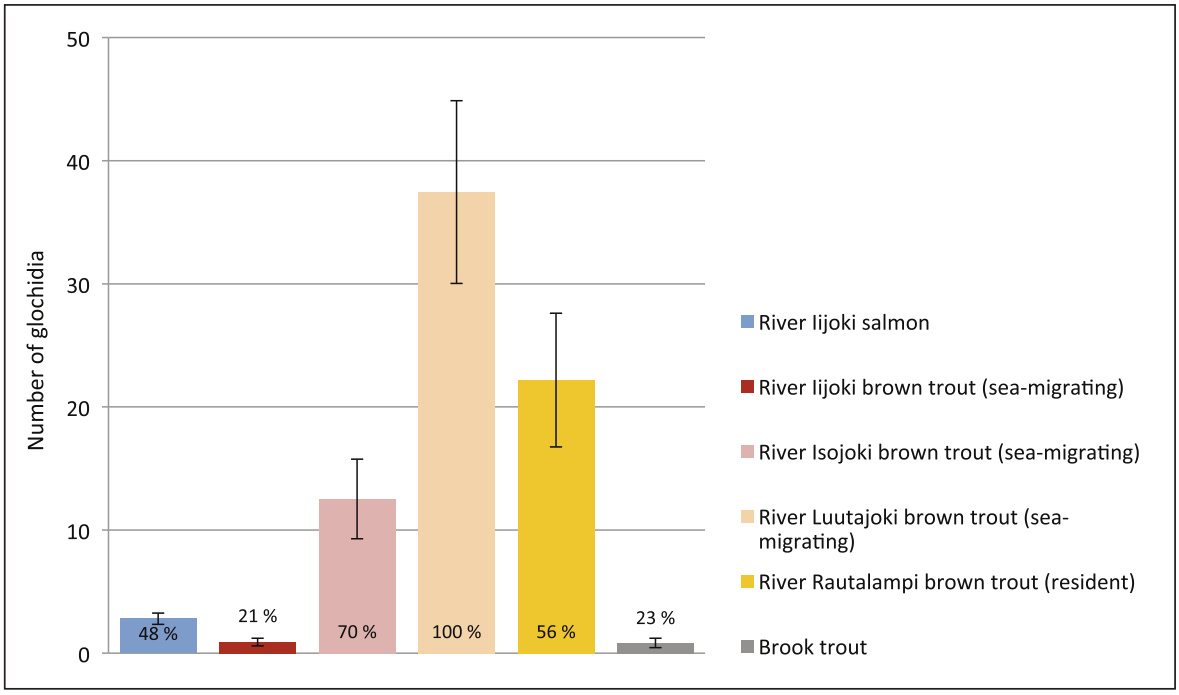

Figure 15. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish and infection prevalence (%) in laboratory infection experiment in 2011 with glochidia from River Koivuoja, which is presumably a sea-migrating brown trout tributary in the River Iijoki catchment. Brook trout and River Iijoki brown trout were the poorest hosts. Interestingly, the non-local Isojoki, Luutajoki and Rautalampi brown trout were the best hosts. Length differences between fish strains existed, but the result did not change when analysed using fish-length-standardized glochidia numbers.

Isojoki, River Luutajoki and Rautalampi strain were better hosts for the freshwater pearl mussel of River Koivuoja than the River Iijoki brown trout or River Iijoki Atlantic salmon, or the brook trout (Fig. 15). In 2011, the size (and age) differences between fish species and strains were unavoidable, but the result did not change even if fish-length-standardized glochidium numbers were used in statistical analyses. Also, it was observed, that brook trout was a very poor host despite the fact that the individuals of that species were the largest fish in the experiment (Fig. 15).

In the 2012 laboratory experiment, the River Iijoki brown trout was clearly the best host for River Jukuanoja freshwater pearl mussel glochidia (Fig. 16). River Jukuanoja is presumably a former home of the sea-migrating brown trout of the River Iijoki catchment. All glochidia larvae excysted and dropped off from River Iijoki salmon and brook trout within 3 months

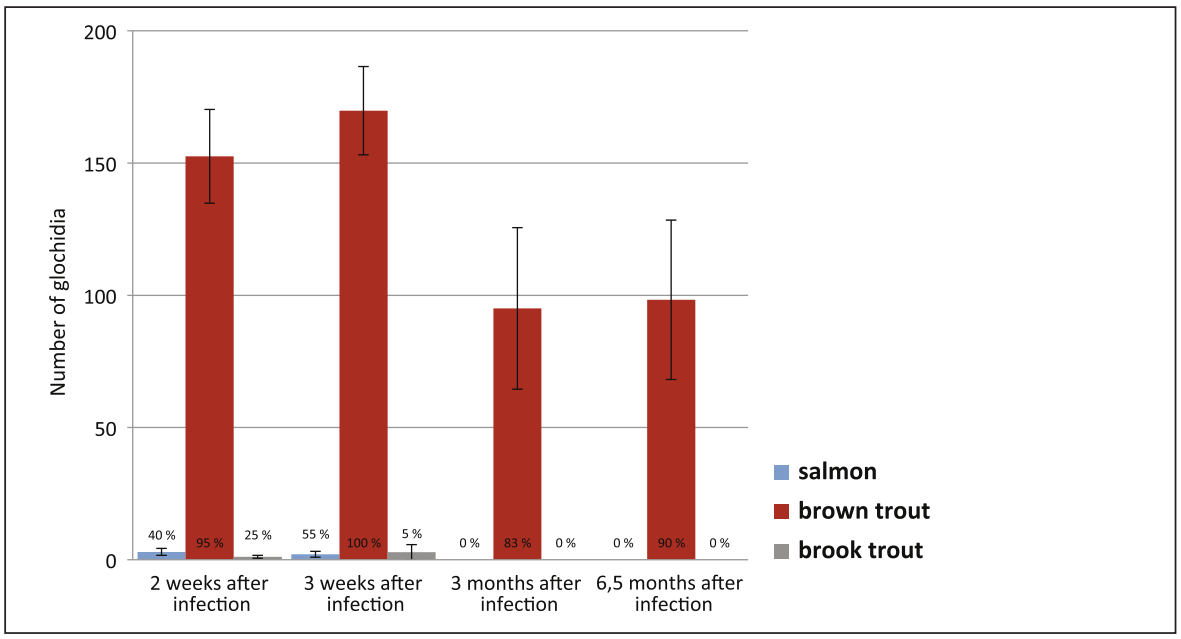

Figure 16. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish and infection prevalence (%) in laboratory infection experiment in 2012 with glochidia from River Jukuanoja, River Iijoki catchment. Salmon and trout mean River Iijoki Atlantic salmon and River Iijoki brown trout, respectively. The River Iijoki brown trout was the best host while the River Ijoki salmon and brook trout were unsuitable hosts.

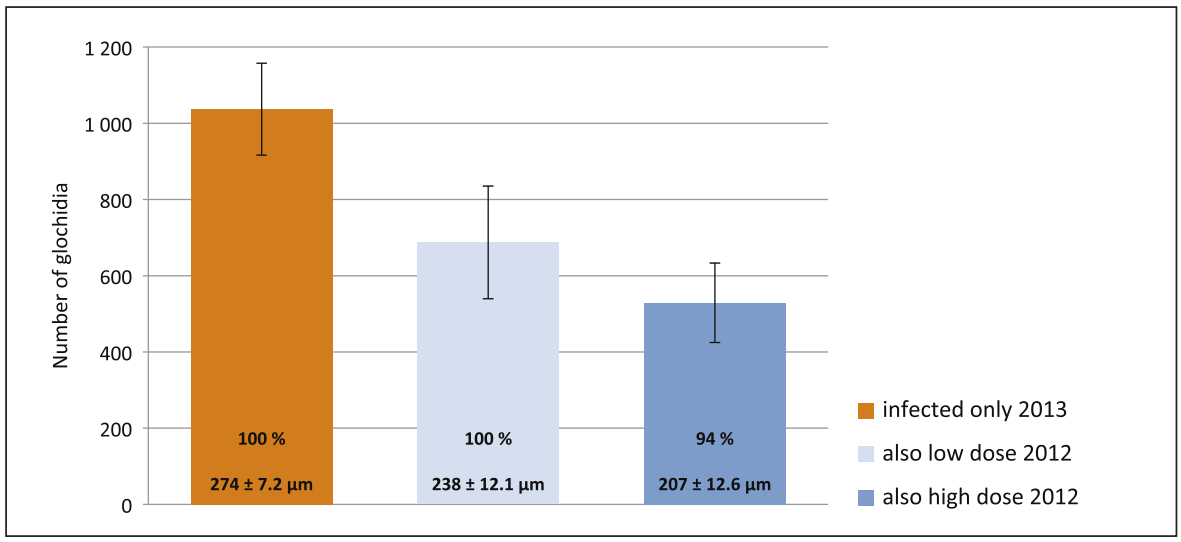

Figure 17. Mean number (± S.E.) of freshwater pearl mussel glochidia per fish, infection prevalence (%) and mean length (± S.E.) of glochidia in different treatments of 1+ year old River Iijoki salmon infected with River Luttojoki pearl mussel glochidia in laboratory experiment in 2013. Mean glochidia abundance and mean glochidium size was lower in fish infected with glochidia in the previous year than in immunologically naïve fish, indicating the development of an acquired immunity in fish against FWPM glochidia.

while 90% of brown trout were still infested 6.5 months after the infection. Both the River Iijoki Atlantic salmon and brook trout seemed to be an unsuitable host for *Margaritifera* of River Jukuanoja. The mean number of glochidia decreased naturally also in the River Iijoki brown trout over time (Fig. 16), but the differences between time points were not statistically significant. The growth rate of larvae was also followed, and larvae were observed to grow from about 70 µm to 250–300 µm during the 6.5 months of the experiment.

4.5.2 Laboratory experiments in 2013; immunization and salmon-trout comparison of River Luttojoki glochidia

In the immunization experiment, 100% of the immunologically naïve, 1+ year old River Iijoki Atlantic salmon were infected with very

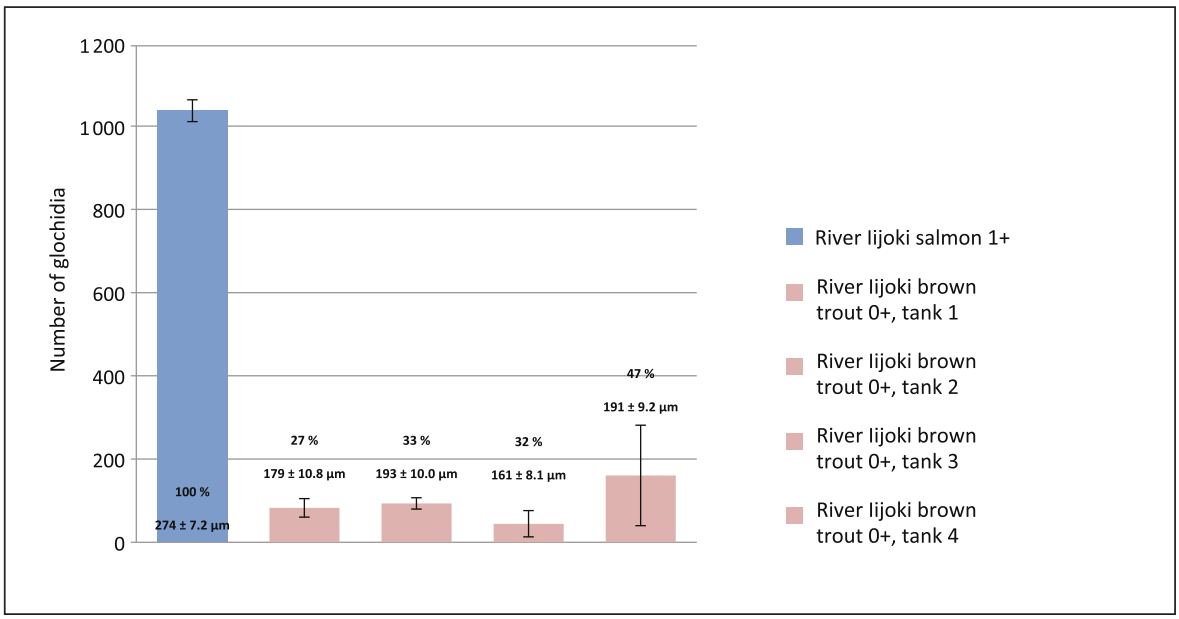

Figure 18. Mean number (± S.E.) of freshwater pearl mussel glochidia per, infection prevalence (%) and mean length (± S.E.) of glochidia in 1+ years old River Iijoki salmon (bar on the left) and in four different tanks of 0+ River Iijoki brown trout infected with River Luttojoki FWPM glochidia in the laboratory experiment in 2013. Mean glochidia abundance and size of glochidia was clearly lower in brown trout than in salmon, suggesting that glochidia of River Luttojoki FWPM developed better in salmon than in trout.

high numbers of glochidia (Fig. 17) originating from the main channel of River Luttojoki, the previous spawning area of Atlantic salmon. Those River Iijoki salmon that were infected with a low dose of pearl mussel glochidia one year earlier were also 100% infected. On the other hand, among salmon infected with a high dose of glochidia a year before, the prevalence of infection was slightly lower, suggesting that some aqcuired immunity migth have developed. Moreover, the number of glochidia was much lower in those fish, which were infected a year before (Fig.17). Also, the size of larvae indicates acquired immunity: larvae developed fastest in naïve fish and slowest in fish which were exposed to high dose of pearl mussel larvae earlier (Fig. 17).

In comparison, only 27–47% of 0+ River Iijoki brown trout became infected by River Luttojoki glochidia. The numbers, prevalences and sizes of larvae in trout (Fig. 18) were in every tank much lower than in any of the salmon groups (Fig. 17), even though both salmon and brown trout were infected with the same batch and a similar dose of glochidia. This suggests that glochidia of the River Luttojoki pearl mussels develop better in Atlantic salmon than in brown trout.

5 Cultivation experiments

5.1 Methods

5.1.1 Development of freshwater pearl mussel glochidia and excystment of juvenile mussels

To study the development, collection and cultivation of small pearl mussels, 150 0+ year class brown trout (River Iijoki stock, from Ohtaoja fish farm) were infected with freshwater pearl mussel glochidia from River Jukuanoja (Iijoki catchment) on 28 August, 2012 and kept over winter at the Konnevesi research station. After 16 days, five fish were examined, and were found to be carrying hundreds of glochidia per fish. 8.5 months after infection, monitoring of the excystment and drop-off of juvenile mussels from fish was commenced. Artificial heating was applied to the tank starting on 26 April, 2013. In late April, so as to induce juvenile drop-off, the temperature was increased rapidly to 11–12°C, while the normal incoming water was about 3°C. After this initial peak, the temperature was gradually decreased. On 5 May, the temperature was 6.6°C higher and on 1 June and 2.9 °C higher than the normal incoming water.

On 26 June, the heating was finally stopped. A 'collection box' (30 x 20 x 15 cm) was made from a small mesh size (20 µm) net and set into the outlet of the tank to retain the juvenile mussels (Fig. 19). The juvenile pearl mussels were collected from the box, counted and measured for size by light microscope at least twice a week during the period 13.05.–12.07.2013. For collecting juvenile mussels, the box was gently shacked and water was directed to one corner of the box. Then a syringe was used to collect the water containing the juveniles. In addition to the collection box, siphoning was applied to collect juvenile mussels from the bottom of the tank. Each time, 10 l of water was collected by siphon from the bottom of the tank and 0.5 l by syringe from collection box. Moreover, the gills of five randomly selected fish were observed twice per week with the naked eye so as to verify that *M. margaritifera* glochidia were dropping off.

5.1.2 Infection of fish on a fish farm

To test the applicability of infecting juvenile salmonids at a fish farm (in order to have pearl mussel glochidia in the fish that will be stocked in the rivers), River Iijoki salmon and River Iijoki brown trout were infected with glochidia at the Vääräniemi Oy fish farm in the autumn of 2012. The fish farm is located in the Iijoki catchment and provides salmonids for different stocking programmes in the Iijoki area. Infection of River Iijoki brown trout took place on 30 August. Pearl mussel glochidia were collected from River Jukuanoja on 30.08.2012 (water temperature 13.0°C) by placing 15 mussels in individual buckets with 7 l of river water for 30 minutes. The glochidia suspension was transported to the Vääräniemi fish farm where it was emptied into the 3 m^2 trout tank where the water level was lowered to 40 cm, the water flow stopped and extra aeration provided during the 0.5 hour exposure. Another 15 mussels were collected on 3 September (water temperature 11.8°C) to infect 500 Atlantic salmon at Vääräniemi fish farm using the same method as above for trout.

Figure 19. Collection of *Margaritifera* juveniles from the outflowing water by trainee Motiur Chowdhury. The glochidia-bearing brown trout are in the upper tank while the 'collection box' is floating in the lower tank. Juveniles collected were planted into a growing tank with sand on the bottom, where their growth will be monitored after the project.

5.1.3 Investigation of the success of juvenile Margaritifera *stocking carried out in 2007*

In the summer of 2007, approximately 20,000 pearl mussel juveniles were planted in River Ala-Haapuanoja, in the River Iijoki catchment. The juveniles developed in brown trout at the West Finland Environmental Centre Kokkola. On 16–17 September, 2013, the river was re-visited and studied for young pearl mussels (water temperature 13.0°C). Study sites were (1) directly below the stocking site, (2) 2,000 m down from the stocking site, and (3) 100 m above the stocking site. On average an 8 cm surface layer (range 4–12 cm cm) of the bottom sediment was excavated from 0.25 m^2 quadrats placed randomly on the river bottom. The sediment was then sieved on site with a series of hand sieves of mesh sizes 19 mm, 12 mm, 5 mm and 2.5 mm (Fig. 20). Locations and numbers of the quadrats were as follows.

(1) Directly below the stocking site, two river sections: a) exactly from the stocking site (10 quadrats, 2.5 m^2), b) 50 river metres down from the stocking site (10 quadrats, 2.5 m^2).

(2) 500 m down from the stocking site, two river sections separated by 50 m, a) 12 quadrats, 3.0 m^2 and b) 12 quadrats, 3.0 m^2.

(3) Control site 50 m up from the stocking site, one river section (12 quadrats, 3 m^2).

River sections sampled were 10–30 m in length.

For methodological calibration, two 50 m sections located 1 km apart from the reference river, River Haukioja, Iijoki catchment, known

Figure 20. Series of sieves (mesh size 19 mm, 12 mm, 5 mm and 2.5 mm from left to right) and sediment excavation tools used in juvenile pearl mussel search in the River Iijoki area in September 2013.

to have juvenile *M. margaritifera*, was sampled on 17 September, 2013, using the same method as above.

5.2 Results of cultivation experiments

5.2.1 Development of freshwater pearl mussel glochidia and excystment of juvenile mussels

The total catch was 339 juvenile mussels, of which the great majority, 330 individuals (97.3%), was caught from the out flowing water (collection box). Only 9 juveniles (2.7%) were caught from the bottom of the fish tank. Release of juveniles from host fish was observed throughout the monitoring period, starting as early as 9 May, but peak drop-off took place on 24 June, probably as a response to increased water temperature (Fig. 21). To analyse dependence between temperature change and juvenile release from fish, the monitoring period was categorized into periods of temperature increase and periods of temperature decrease. Periods of cooling temperatures were 8–20 May (from 11.6 to 9.7°C), 4–17 June (from 10.5 to 9.5°C) and 25–27 June (from 11.2 to 9.5°C). Periods of increasing temperatures were 21 May–3 June (from 9.7 to 10.6°C), 18–24 June (from 9.5 to 11.2°C) and 28 June–29 July (from 9.5 to 13.4°C) (Fig. 22). The mean number (± s.e.) of juveniles dropped off from fish during cooling and warming periods were 1.9 ± 0.4 and 9.9 ± 1.9 individuals per day, respectively, the difference being statistically significant (ANOVA, Log-transformed data, $F_{1, 45} = 18.021$, $P < 0.001$). The size of released juveniles increased throughout the monitoring. In early May, the length of juveniles was around 300 µm, but in late July more than 400 µm (Fig. 22). The sum of day-degrees from 24 April to the main peak of juvenile shedding, 24 June, was 394°C. To the first signs of juvenile drop-off, 28 May, the sum of day-degrees was 182°C.

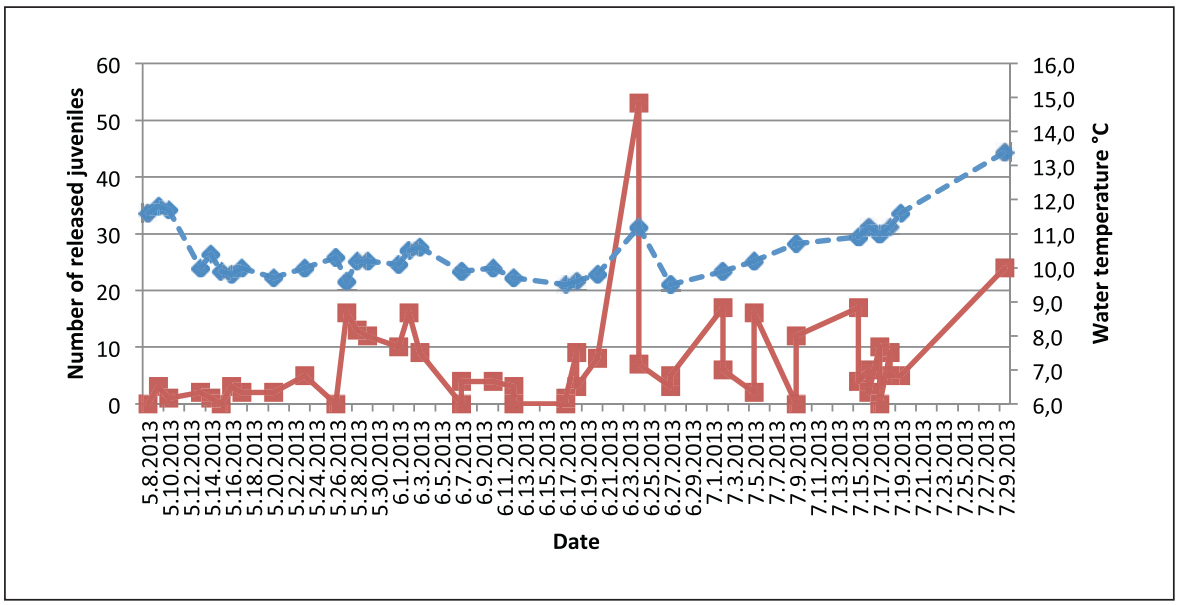

Figure 21. Daily catch of juvenile *Margaritifera* (solid line) and water temperature (dashed line) during the juvenile drop-off monitoring at the Konnevesi Research Station 2013. Peaks of juvenile release from fish coincided with periods of increasing temperature. Artificial heating was applied to the tank from 26 April to 26 June, 2013, so that on 5 May the temperature was 6.6°C higher and on 1 June, 2.9°C higher than the normal incoming water.

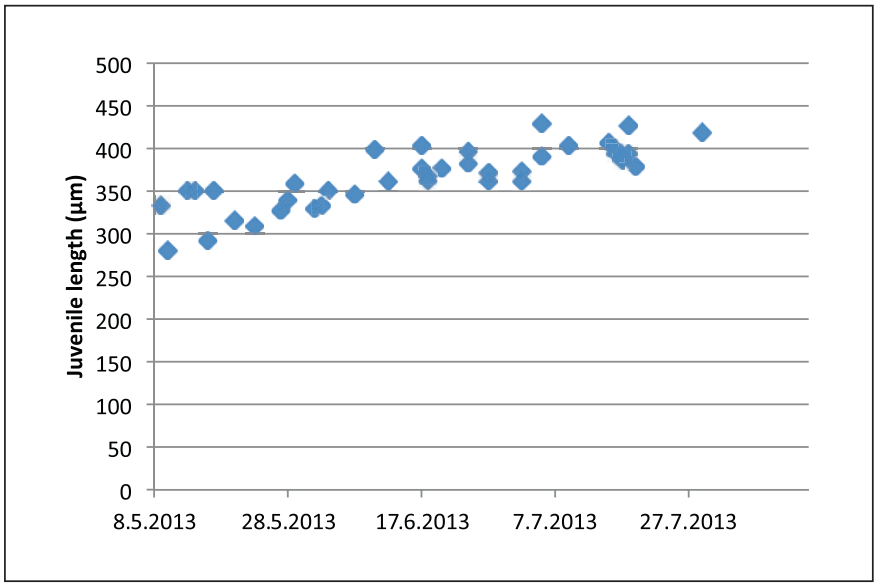

Figure 22. Mean length of released juvenile *M. margaritifera* during the juvenile drop-off monitoring at the Konnevesi Research Station 2013.

5.2.2 Infection of fish in fish farm

In the fish farm experiment, the first infection with River Iijoki brown trout resulted in a very great abundance of glochidia. The infection was so heavy that the fish started to succumb and die. Therefore, the trial was terminated on 3 September, 2012 and five of the fish that had survived were studied for glochidia. Minimum and maximum numbers of glochidia in fish studied four days post infection were 3,296 and 6,480 larvae respectively, with an average number of 4,588 glochidia (n = 5, fish length 75–84 mm). Unlike the brown trout, the infection of River Iijoki Atlantic salmon resulted in a low abundance of infection. Seven days after infection the mean abundance of infection was 298 glochidia per fish (min-max, 39–537, n = 6, fish length 59–90 mm). On average 49 glochidia per fish (one sixth of larvae) was loose, not properly attached to the gills of salmon. Such loose glochidia were not observed in brown trout.

5.2.3 Investigation of the success of juvenile Margaritifera *stocking carried out in 2007*

In sediment excavation and sieving at River Ala-Haapuanoja, the ten 0.25 m^2 quadrats precisely at the 2007 stocking site revealed no *M. margaritifera* juveniles. However, one juvenile mussel was discovered in one of the ten 0.25 m^2 quadrats, 50 m below the stocking site. The individual observed was 9 mm in length, with three growth rings (Fig. 23). Thus, if the umbo area is expected to cover four years, the age of the juvenile could be estimated as 7 years. In that case, the juvenile pearl mussel found could have originated from the 2007 stocking. The combined catch below the Ala-Haapuanoja stocking site was one juvenile per 5.0 m^2 (2.5 + 2.5 m^2 in total), 0.2 individuals per m^2. A conservative estimation of potential, gravel-dominated, suitable juvenile pearl mussel area in the 80 m river section covered by the juvenile search, starting from the stocking site, was 120 m^2. Thus, it is possible that more juveniles could be found with a greater effort. Excavation of the 12 + 12 quadrats 500 m down from the 2007 stocking site did not reveal any juvenile mussels. In addition, the control section 50 m up from the stocking site (12 quadrats) had no juvenile *M. margaritifera*.

Figure 23. A juvenile pearl mussel individual found from River Ala-Haapuanoja (Iijoki catchment. The individual was found 50 m down from the site where 20,000 small 0 year old juvenile mussels were stocked in 2007. The mussel was 9 mm in length and 7 years in age, thus probably originated from the 2007 stocking.

A total of 21 quadrats (5.25 m^2) were studied from the reference river, River Haukioja. Five of the quadrats (23.8%) were occupied by small mussels; 1 x quadrat with two juveniles (both in the size class 30–34 mm), 3 x quadrats with two juveniles (both in the size class 35–39 mm), and 1 x quadrat with 15 juveniles (all in the size class 35–39 mm), 23 juveniles altogether. Thus, the mean juvenile density in River Haukioja was 4.4 individuals per m^2, but the juveniles were highly aggregated.

6 Discussion

6.1 The freshwater pearl mussel is adapted to different fish hosts in different rivers

The present results of caging and laboratory experiments show that freshwater pearl mussels prefer different host fish in different waters. In bigger streams, to which Atlantic salmon earlier migrated, such as River Simojoki, River Luttojoki and River Livojoki, the freshwater pearl mussel preferred Atlantic salmon as a host instead of brown trout. Also, the growth rate of glochidia was faster in salmon. It is worth noting that in the present study, the salmon dependence of *M. margaritifera* was observed in salmon rivers flowing to the Baltic Sea (River Simojoki and River Livojoki) as well as into the Arctic Ocean (River Luttojoki). Also, in Norway a number of rivers have been characterized as almost exclusively parasitizing either Atlantic salmon or brown trout, even when both hosts are present (Larsen & Karlsson 2012). These results suggest that adaptation to using salmon as a host in salmon rivers may be a common phenomenon. In line with this, in the present project a genetic differentiation between freshwater pearl mussels originating from salmon rivers and brown trout rivers was observed (Annex D), as well as by Karlsson et al. (2013b) in Norwegian *M. margaritifera* populations. Considering the conservation of *M. margaritifera*, this is an important finding. It emphasizes (1) the importance of maintaining the remaining salmon populations and their spawning migrations, and (2) the importance of restoring the lost salmon stocks and rivers, including free migration from the sea to the spawning grounds. This result also

emphasizes that stocking of eggs, embryos or juvenile salmon in River Livojoki, as well as building fishways or transporting adult salmon to their former spawning grounds in the River Iijoki catchment would be highly recommended. However, there is not much time for that, since the mussel population in the Livojoki is already rapidly declining (Annex B). Interestingly, the Rautalampi brown trout, which is the main strain stocked for example to River Livojoki, was the second best host for Livojoki *Margaritifera* after Atlantic salmon.

On the other hand, the current results strongly suggest that *Margaritifera* in rivers occupied by brown trout, either resident or migratory, clearly prefer brown trout over salmon as their host. Only in the year 2011 caging experiments in River Lohijoki and River Koivuoja, there is no statistical difference between salmon and brown trout as host. All other field experiments in brown trout rivers (River Portinjoki 2011, River Porraslammenoja and River Ala-Haapuanjoki 2012), and laboratory experiments with FWPM glochidia from brown trout rivers (River Koivuoja 2011 and River Jukuanoja 2012) indicate better suitability of brown trout over salmon as the host. In these smaller streams the host fish situation is usually not so alarming as in the Livojoki or Luttojoki. In some rivers, a threat for *Margaritifera* recruitment is caused by the invasive species, brook trout, which has recently expanded its distribution range in Finland. Brook trout prefers small streams and is a strong competitor to brown trout, having replaced it in many rivers (Korsu et al. 2007, 2008, Öhlund et al. 2008).According to our results, freshwater pearl mussel cannot successfully use brook trout as a host fish. Therefore, the spread of this species can locally be a serious threat for *Margaritifera* in these waters.

The differences in suitability between different brown trout populations as pearl mussel hosts were not as remarkable as the results between salmon and brown trout. Results on local adaptation of *Margaritifera* were somewhat contradictory. In three cases, the local brown trout was a better host than the non-local strains (field experiments at River Ala-Haapuanoja 2012, and River Hanhijoki and River Kolmosjoki 2013). In two cases, no difference was observed (field experiment at River Lohijoki and River Portinjoki 2011). In one case, the non-local brown trout strain was the better host (laboratory experiment with River Porraslammenoja FWPM glochidia 2011). Thus, the results of the present study do not give 100% clear support for using a local *Margaritifera*-fish host combination, but there are other reasons to favour the original fish and *Margaritifera* populations in pearl mussel rivers and streams. Interestingly, the sea-migrating River Iijoki brown trout was in most cases the worst host. It was a worse host than salmon in salmon rivers, but there was also a tendency towards lower suitability of Iijoki trout among different brown trout stocks in trout tributaries. Even in the possible former sea-migrating brown trout tributaries of River Iijoki, namely River Ala-Haapuanoja, River Porraslammenoja and River Koivuoja, the sea-migrating River Iijoki brown trout was not the best host. The only exception to this pattern was River Jukuanoja, in which the sea-migrating River Iijoki brown trout was proportionally best when compared to the Iijoki salmon. This was clear both in the laboratory experiment in 2012 and in the fish farm infestation trial in which both salmon and brown trout were exposed to glochidia from River Jukuanoja FWPM. River Jukuanoja is also probably a former sea-migrating brown trout river. However, the role of the anadromous River Iijoki brown trout as a host for *M. margaritifera* in the River Iijoki area, especially in comparison to other Iijoki trout populations, should be studied in detail in the future. Signs of local adaptation within the Atlantic salmon rivers were not clear. In River Livojoki such phenomenon was observed in the 2013 field experiment, but not in River Simojoki.

The results of the immunization experiment indicate, that the glochidia infestation does not protect the fish from another infestation later: the prevalence of glochidia infestation among previously infected fish was almost as high than among naïve fish. On the other hand, the acquired immunity was manifested as lower glochidia numbers and slower development rate of glochidia among fish that got the infection second time. Considering the bigger size of the 1+ year class fish (and hence a larger surface area of their gills), they might still serve as good hosts for freshwater pearl mussel even though they

would have been infested by glochidia earlier. However, the development and growth of the glochidia in fish infected second time should be monitored longer than the 3 months in our study in order to ensure the size of the glochidia at the time of their detachment.

6.2 Cultivation experiments

Results of juvenile drop-off monitoring indicate that the development of *M. margaritifera* glochidia can be affected by regulating water temperature. A juvenile collection experiment at the Konnevesi research station in 2013 also revealed that siphoning of the bottom of the host fish tank is not necessary, as the great majority of the juveniles were caught from the outflowing water. In nature, the juvenile drop-off starts between mid-June and early July in the Iijoki catchment rivers (Säkkinen 2012). Thus, the current artificial heating of water resulted in enhanced development and earlier excystment of the glochidia, as the first detached glochidia were observed as early as on 9 May. Therefore, it is possible to regulate the development and metamorphosis of pearl mussel glochidia by changing the temperature in the laboratory. By their size, the juveniles were as big as, or even bigger than, those found in natural rivers of the Iijoki area, around 350 µm at the time of drop-off (Säkkinen 2012). An important finding was that, after the glochidia detachment has started, even a slight temperature increase will trigger metamorphose and excystment of glochidia. The highest drop-off peak of glochidia on 24 June took place after only a 1.4°C increase in water temperature between 18–24 June. This finding can be utilized in controlling the juvenile production; development can be adjusted and timed to achieve maximal juvenile catch for planting purposes, for example. The results also clearly indicate that a decrease in water temperature causes cessation of juvenile excystment and drop-off. Survival and growth of juveniles after the collection is not yet known. The juveniles were planted in buckets with water flow from below, filled with 20 cm of sand/gravel at Konnevesi Research station, where their fate will be monitored in coming years.

Results of the fish farm experiment encourages us to further develop this approach. It is possible to infect the salmon and trout in fish farms before they are stocked to the river. This requires a knowledge of local glochidia release of *M. margaritifera*. It might be good that glochidia are collected and provided by authorized persons. Collection of glochidia in excess does not pay, as too high a dose may harm the fish. Moderate infection rate should be the target, equivalent to high infection rates in the field, some hundreds of glochidia per fish. Attention should be given to suitable host fish. For example, the glochidia of the present study, in River Jukuanoja, very effectively infected brown trout but poorly infected the Atlantic salmon.

Results of River Ala-Haapuanoja sediment excavation and sieving indicate that some of the juvenile *Margaritifera* stocked in 2007 may still be alive.

Annex F

Searching for new freshwater pearl mussel populations

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1 Introduction

Although many of the freshwater pearl mussel (*Margaritifera margaritifera*) populations have been found either accidentally over time or on purpose by active searches, a number of unknown populations probably still exist in northern Fennoscandia. On the other hand, there may be a need to study the fate of the freshwater pearl mussels in rivers or river sections where the species is known to have lived previously, but where the current occurrence is unknown. It is also possible that the freshwater pearl mussel is known to inhabit a certain part of a river while several other sections are unmapped, even though these may provide optimal habitats for freshwater pearl mussel. In addition, monitoring of the status of the freshwater pearl mussel populations may require repeated checking of their occurrence.

Searching for freshwater pearl mussels is usually done by SCUBA diving, snorkelling or by using an aquascope. Dark or turbid water, a stony bottom, aggregated distribution of mussels, low density or some other obstacles may limit usage of the above methods, making them laborious or impossible. An alternative method could be the capture of host fish and examination for pearl mussel glochidia microscopically, or with the naked eye at the site.

For the above reasons, the aim of this work package was to:

1) Develop and test a new search technique, electrofishing method, in which the occurrence of mussels is investigated by studying the gills of host fish for the parasitic glochidium larvae of freshwater pearl mussel, and to

2) Search for new, previously unknown freshwater pearl mussel populations in the northern areas of Finland and Norway

2 Study areas and methods

The study and search areas are located in Lapland, in the River Näätämö (In Norwegian Neiden) and River Teno (In Norwegian Tana) water systems, and in North Ostrobothnia, in the River Iijoki catchment. First in 2011–2013 the Centre of Economic Development, Transport and the Environment (ELY Centre) of Lapland conducted electrofishing survey in 44 rivers in the water system of the Näätämö and Teno rivers and in one location in the Paatsjoki water system (Fig. 1). A total of 72 test areas were examined, with surface areas varying from 4 to 527 m^2. The combined surface areas of the test areas was 7,373 m^2.

In Iijoki river basin, a total of 78 sites in 50 rivers of River Iijoki catchment were electrofished during 2011–2013 by the Natural Heritage Services, Metsähallitus, and the University of Jyväskylä (Figs 2–3). Electrofishing time varied from 15 minutes to 1 hour 30 minutes per site on each occasion. Some sites were electrofished on two different dates. The fish caught were identified and measured for length. Gills of salmonids were inspected for the occurrence of pearl mussel

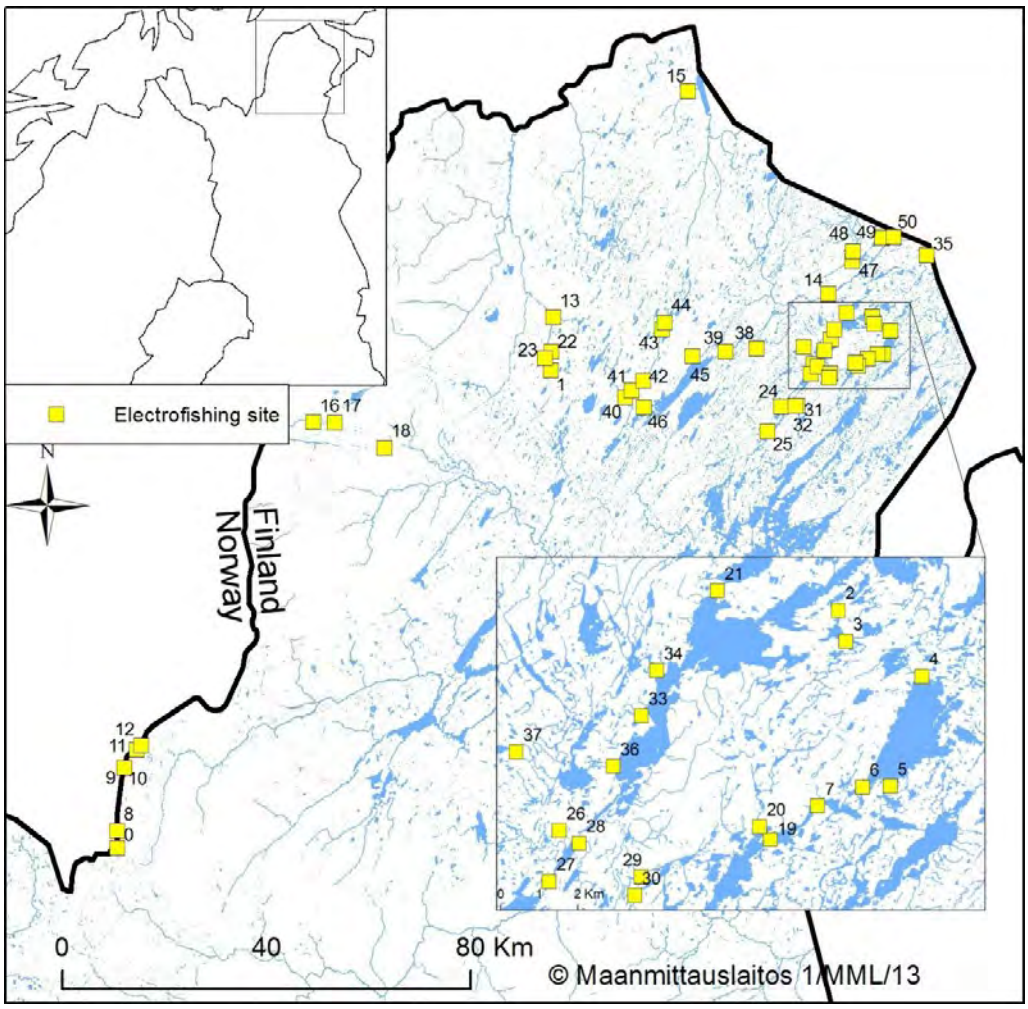

Figure 1. Map of electrofishing sites in Lapland, in the River Näätämö (Neiden) and River Teno (Tana) water systems. © Metsähallitus 2015, © National Land Survey of Finland 1/MML/15.

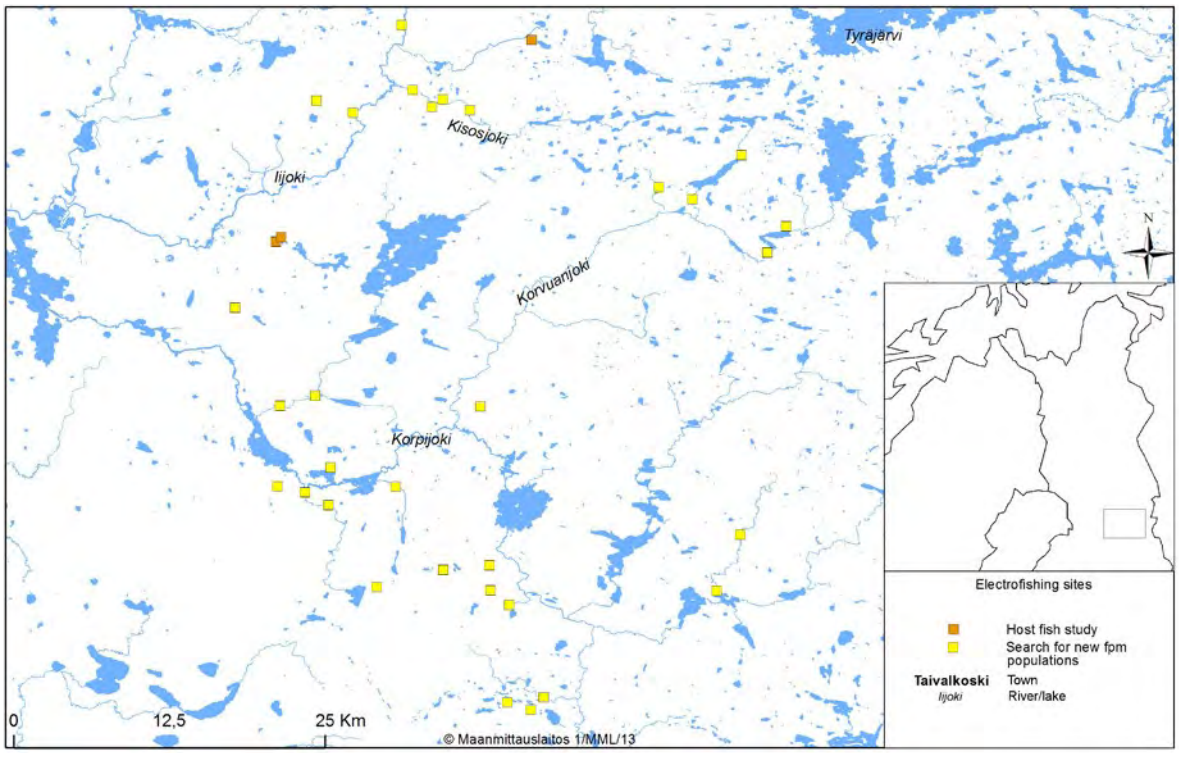

Figure 2. Map of electrofishing sites in the southern River Iijoki catchment. © Metsähallitus 2015, © National Land Survey of Finland 1/MML/15.

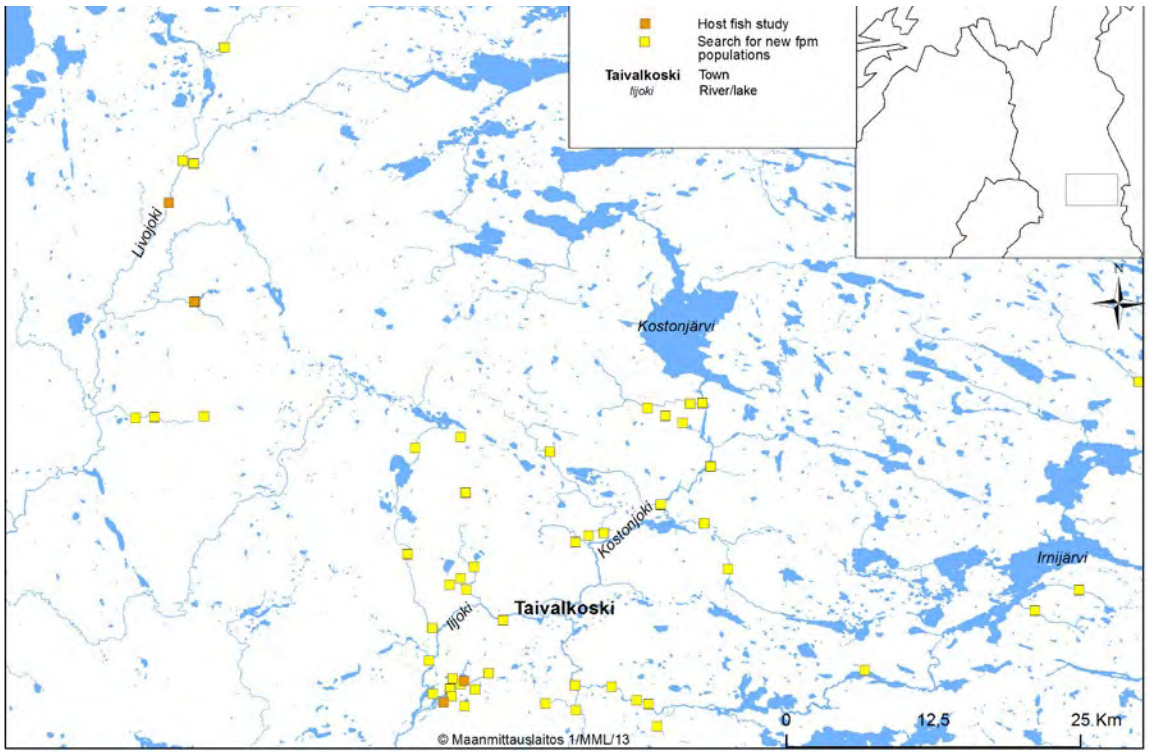

Figure 3. Map of electrofishing sites in the northern River Iijoki catchment. © Metsähallitus 2015, © National Land Survey of Finland 1/MML/15.

Figure 4. Gills of young brown trout infected with a high number of 'old' freshwater pearl mussel glochidia. Individual glochidia can be seen as the small whitish granules among the red gill lamellae. River Hanhioja (Inari), July 2011. Photo Marko Kangas.

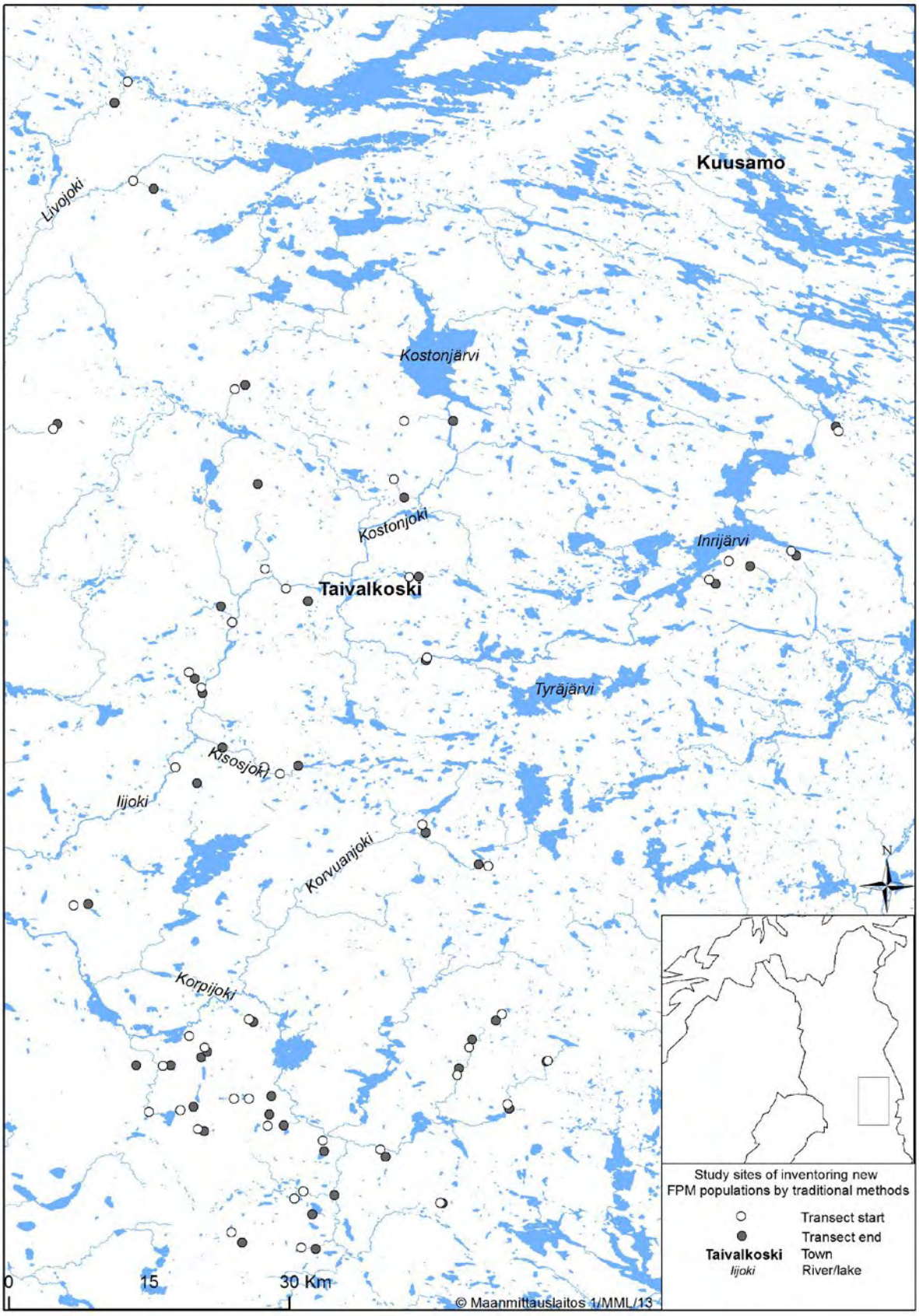

Figure 5. Map of sites surveyed using traditional methods (aquascope, snorkelling, SCUBA diving) in the River Iijoki catchment. © Metsähallitus 2015, © National Land Survey of Finland 1/MML/15.

glochidia with the naked eye (Fig. 4). If the catch was large enough, a subsample of 1–5 salmonids was killed, stored on ice and transported to laboratory for a microscopic examination

For comparison, pearl mussel inventories using traditional methods (aquascope, snorkelling, diving) were carried out in 2011–2013 on in 40 tributaries of River Iijoki catchment, and in 27 rivers in River Näätämö and River Teno catchments (Figs 5–6). More detailed descriptions of the large field surveys performed in the River Näätämö/Teno and River Iijoki water systems are given separately in chapters 4 and 5.

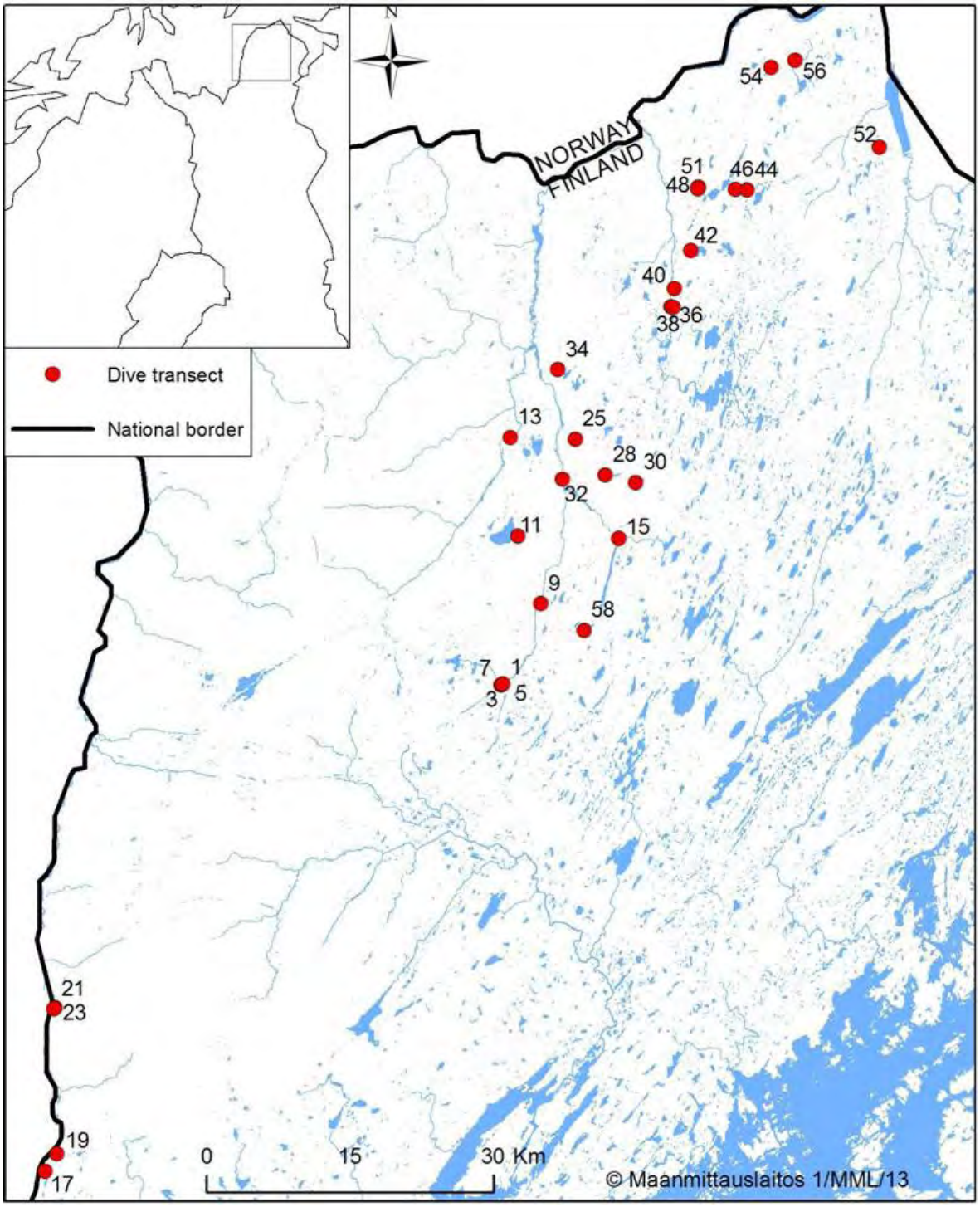

Figure 6. Diving sites in the Näätämö and Teno catchment areas. © Metsähallitus 2015, © National Land Survey of Finland 1/MML/15.

3 Results of the suitability and reliability of electrofishing method in finding freshwater pearl mussels

In spring and early summer 2011 in the River Jukuanoja, River Iijoki catchment, the infection status (infected/uninfected) assessed by the electrofishing method with a quick dip in the field was 17 fish out of 18 (Table 1). In spring and early summer 2011 in River Koivuoja, the result 17 out of 22 fish. Most importantly, in spring and early summer, no false positive records were achieved, and the field-assessment was 100% correct in all cases in which the real number of glochidia per fish was at least 20 (Table 1). On the contrary, when the electrofishing was done in the autumn, the infection status was scored 'uninfected', even though the fish were infected by freshwater pearl mussel glochidia, as seen in the case of River Jukuanoja, 2011 (Table 1). This indicates that the new, freshly attached glochidia cannot be observed with the naked eye in the autumn.

In 2012 the electrofishing method was tested in River Majovanoja and River Pahkaoja, in the River Iijoki catchment, so that three independent observers conducted the glochidiosis intensity scoring in the field (Table 2). Repeatability of the scoring between the observers was usually reasonably good, although the scoring by the experienced observer was most frequently closest to the real glochidia number (Table 2). The mean field scores by the three observers correlated statistically significantly with the real number of glochidia in (Fig. 7). Thus, in addition to the occurrence of pearl mussel glochidia, also the estimation of the number of glochidia can be achieved by a quick dip with the naked

Table 1. Field scores of glochidiosis (infected or uninfected with *M. margaritifera*), fish length and real numbers of glochidia (Glochi) in electrofished brown trout from River Jukuanoja and River Koivuoja of the River Iijoki catchment on different dates between June and August, 2011. Incorrect glochidiosis scores with the naked eye on site in the field are marked in red. 'Old glochidia', 300–400 µm in length, attached to host fish in previous autumn, were reliably visible to the naked eye at the site. 100% correct classification with the naked eye was achieved when the number of glochidia per fish was greater than 20. 'New' freshly encysted, small 70 µm glochidia in August were not visible to the naked eye.

2011 Test	River Jukuanoja, 4.7 km			River Koivuoja, 14.7 km		
	Fish mm	Field score	Glochi	Fish mm	Field score	Glochi
Old glochidia						
June 9	77	infected	176	134	infected	112
	109	infected	165	151	infected	67
	147	infected	241	113	infected	558
	77	no	0	160	infected	1674
	78	infected	105	-	-	-
June 28	94	infected	16	162	no	3
	132	infected	1460	77	infected	108
	110	infected	406	139	no	19
	90	infected	126	138	no	1
	-	-	-	97	no	0
July 20	115	no	11	158	no	1
	112	infected	6	169	no	1
	168	no	0	86	no	0
	54	no	0	146	no	0
	128	infected	128	-	-	-
August 3	216	no	0	138	no	0
	112	no	0	150	no	0
	63	no	0	157	no	0
	59	no	0	106	no	0
Total	n=18			n=17		
Correct score	17	94 %		12	71 %	
New glochidia						
August 32	121	no	16	148	no	0
	126	no	265	143	no	0
	71	no	235	66	no	0
	134	no	329	145	no	0
	-	-	-	65	no	0

Table 2. Field scores of intensity of glochidiosis, scored from 1 to 5 (1=low, 5=high intensity) by three independent observers (1–3), observer 1 being experienced. The study was carried out in River Majovanoja and River Pahkaoja in the River Iijoki catchment in June 2012. Incorrect scores are marked in red.

2012 Test	Fish mm	Field scores by three persons				Glochi
June 5-8		1	2	3	Mean	
River Majovanoja	69	0	3	0	0.33	4
13.5 km	68	3	3	2	2.67	141
	70	3	0	4	3.33	143
	144	0	0	0	0	0
	95	0	0	0	0	0
	120	0	0	0	0	0
	106	0	0	0	0	0
	82	0	0	0	0	0
	109	0	0	0	0	0
	107	0	0	0	0	0
	94	0	0	0	0	0
River Pahkaoja	75	1	1	1	1.00	14
4.4 km	122	3	3	2	2.67	399
	92	1	1	1	1.00	23
	57	0	1	0	0.33	0
	52	0	1	0	0.33	0

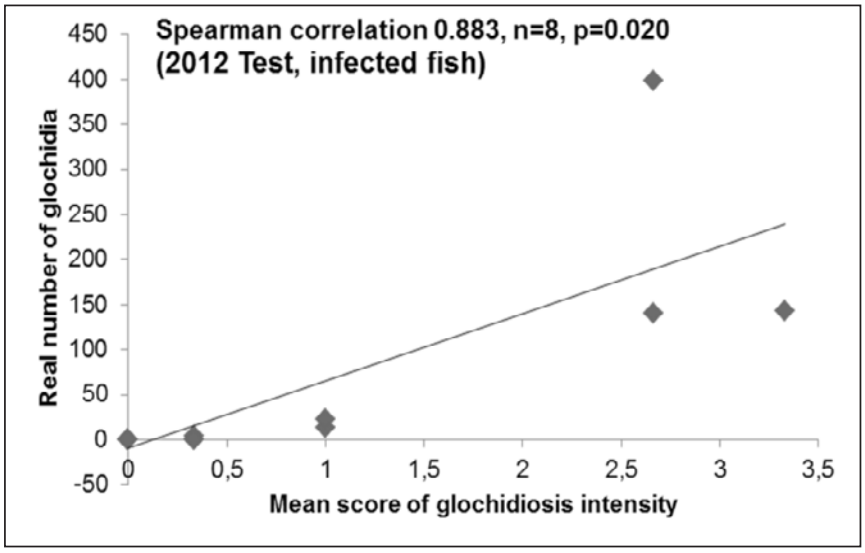

Figure 7. Correlation between the mean field scores of intensity of glochidiosis, scored from 1 to 5 (1=low, 5=high intensity) by three independent observers, and the real number of glochidia larvae. The study was conducted in River Majovanoja and River Pahkaoja in the River Iijoki catchment in June 2012.

eye in the field when using the electrofishing method.

In spring and early summer 2012, the electrofishing method was tested in the River Iijoki catchment within a) tributaries with an unknown pearl mussel and brown trout status, and, for comparison, within b) tributaries where it is known that pearl mussels do not occur, but with an unknown brown trout status. In this test that included 29 tributaries, 4.3–18.3 km in length, in 9 tributaries in which the fish catch was between 2 and 11 brown trout, the correct classification of the tributary (infected vs. uninfected) was achieved for 100% of the tributaries when the field observations with the naked eye were compared to laboratory examination results of the fish collected (Table 3).

Table 3. Application of the electrofishing method to 29 tributaries of the River Iijoki catchment in 2012, in tributaries with unknown brown trout status and unknown/known pearl mussel status. If the fish catch was of a reasonable size (between 2 and 11 individuals), the classification of the river as pearl mussel infected vs. uninfected was 100% correct. Three new, previously unknown *M. margaritifera* populations were found by using the electrofishing method in the River Iijoki catchment in 2012.

2012 Application of fishing method to 29 rivers						
Mussel status	Trout status	n rivers	n fish per river	Rivers scored infected	Rivers scored uninf.	Correct score
Unknown	Unknown	9	2-11	3	6	100 %
Unknown	Unknown	4	1	2	2	50 %
Unknown	Unknown	11	0	-	-	-
No mussel	Unknown	5	3-6	0	5	100 %
→ fishing revealed three new *Margaritifera* populations						

Of those 9 tributaries, 3 were found to contain freshwater pearl mussel. Thus, the testing of the electrofishing method in 2012 revealed 3 new, previously unknown pearl mussel populations in the River Iijoki catchment. The result was later verified by aquascope search. If only one individual fish was caught by electrofishing, the classification of the tributary was unreliable, only 50% correct. In addition, almost one third of the tributaries were such that the brown trout catch was zero (Table 3). In all of the 5 tributaries with a previously unknown status of pearl mussels, the electrofishing method revealed that the host fish, brown trout, was around, but the fish were not infected by pearl mussel glochidia. This was shown to 100% by laboratory examinations (Table 3). It is worth noting, that the search of those 29 rivers by electrofishing took two weeks and resulted in three new pearl mussel populations. It can be estimated that the same search by aquascope, snorkelling and diving would have taken much longer.

4 Surveys of freshwater pearl mussels in the Näätämö and Teno river basins 2011–2013

4.1 General description of the survey area

The catchment area of the area being surveyed covers about 19,041 km², of which the Teno River catchment area is about 16,098 km², and that of the Näätämö River is 2,943 km². The survey area in its entirety is part of the natural geographical area of the Fennoscandian Shield. A small part of the Teno River on the Norwegian side is part of the natural geographical area of the Caledonian Mountain Range.

Moraine is the most common soil type in the Teno-Näätämö River catchment, as is the case in other parts of Northern Finland as well. The ground cover is thin in many places and rock exposure often occurs. Ridges and deltas have been formed in the valleys (i.e. the Teno River and Utsjoki River valleys). In addition, the river has, over time, amassed big layers of sand, where it has later carved out river terraces at different elevations, which serve as agricultural and residential areas.

Most of the bedrock of the Teno area comprises the same geological formations as that in the rest of Northern Finland: granite gneiss, shale, and deep-seated rock areas. Acidity is a typical feature of the bedrock, and this is reflected in the soil and also in the vegetation as a lack of demanding species.

The waterways in the catchment area are mainly oligotrophic, clear, or containing small amounts of humus. Lakes with a surface area of more than 5 km² are of the low-humus type. The river catchment areas are mostly moorland, and of the rivers with catchment areas of more than 200 km² only Vaijoki is classified as a peat soil river. In the survey areas, there are areas with great differences with respect to their sensitivity to acidification. The waters of the survey area are mostly relatively sensitive to acidification, as the weather decay of the predominantly granite and gneiss areas is slight.

4.2 Survey methods

4.2.1 General survey methods

Electrofishing is a method which has been developed for estimating populations of young salmonid fish in flowing waters (Fig. 8). It is an efficient way to catch young salmon and trout up to three years of age in shallow (< 0.8 m) and sheltered parts of waterways.

Electrofishing devices comprise a source of current (a generator or a battery), a transformer, and two electrodes – an anode (+) and cathode (-). Catching the fish by electrofishing is based on a direct current field in whose range a difference in tension is formed between the head and tail of the fish. When the fish comes close enough to the electrode (anode), the difference in tension causes a so-called forced swimming reaction in the direction of the anode, and when the fish is at a distance of about 0.3–1 metre of the anode, the fish is stunned and easy to net for research. Electrofishing rarely causes damage to the fish that are caught, making it an excellent way to catch young fish.

Electrofishing was used in the initial survey of freshwater pearl mussel populations, and was based on finding of salmonids with the freshwater pearl mussel glochidia.

Figure 8. Electrofishing provides good information on fish species in the water systems. Photo Marko Kangas.

4.2.2 Phases of the work

The first phase of the survey was an examination of the map from which the most likely areas of distribution of the freshwater pearl mussel in the water system were selected. Next, the most suitable areas for electrofishing (moderately fast flowing sections) were surveyed. The electrofishing points were numbered and a fishing/inventory sequence was drafted for the points/test areas.

Work in the field was the second phase of the surveys. All areas under investigation were inventoried by drafting a so-called "rough" habitat description. In addition to the above, the areas were photographed, their coordinates set, and they were marked on a map. Fish caught using the electrofishing method were examined. All of the fish were inspected visually (condition of the fins, wounds, etc.). The trout and salmon that were caught were measured, their gills were inspected for glochidia, and they were photographed.

All of the electrofishing took place in June with the exception of 2011, when the fishing was conducted in the first week of July. The early summer was chosen as the time for fishing, because that is when the glochidia of the freshwater pearl mussels are easily discerned in the gills of the fish without special equipment (microscopes).

The early summer of 2013 was very warm, and at that time the temperature of an unnamed stream that flowed into Lake Vainosjärvi was measured at 19.6°C (7 June 2013) and the temperature of the River Vainosjoki, which flows out of Vainosjärvi was 15.2°C (8 June 2013). These high temperatures meant that part of the inventories of the Centre of Economic Development, Transport and the Environment of Lapland were conducted by diving, using a dry suit, a snorkel, and mask.

The following was recorded in connection with the diving method: a rough habitat description, photographs from the location, fish species that were observed, the coordinates of the start and finish points, other observations worthy of note, freshwater pearl mussels present/absent.

This work (electrofishing and diving methods) was made more demanding by the wilderness nature of the study area. Owing to the large amount of research equipment and the wilderness character of the water areas, it was necessary to spend long periods in the field

without interruption. All transport was either on foot or by canoe along water routes. A seaplane was used to reach the most remote locations. The main focus of this survey was on the tributaries of the Näätämö and Teno rivers.

4.3 Results

Locations of electrofishing surveys are listed in Appendix 1. Trout or salmon were caught in 28 out of 44 streams and rivers in Lapland, in the catchments of River Näätämö, River Teno and River Paatsjoki. Salmon and trout measuring less than 10 cm were caught in 15 different rivers or streams.

No glochidia of freshwater pearl mussels were found in the gills of the fish. Some inflammation of the gills was seen in young salmon in the Näätämö River and Utsjoki as well as Gálddašjohka in June 2013. This observation could suggest that the fish may have previously had glochidia, which had already detached from their host fish. The exceptionally high water temperature for the time, combined with the electrofishing method/the anaesthetising of the fish might have caused haematomas in the gills of the young fish. Inflamed gills were not seen in fish in other areas.

By diving, a total of 3,885 metres of riverbed were surveyed. No new freshwater pearl mussel populations or remnants of their shells were observed in the surveys. Brown trout or Atlantic salmon were observed in 17 different rivers/streams. The diving sites are shown in Appendix 2.

5 Surveys of freshwater pearl mussels in the River Iijoki water system in 2011–2013

5.1 Results

Rivers surveyed by electrofishing are given in Appendix 3. The total number of tributaries investigated by electrofishing was 38. Rivers surveyed by traditional methods (aquascope, snorkelling, SCUBA diving) are given in Appendix 4.

New, previously unknown freshwater pearl mussel populations were found by the electrofishing method from three out of the 38 rivers surveyed. With traditional methods (aquascope, snorkelling, diving) six new, previously unknown populations were found in 40 tributaries surveyed. Thus, the total number of freshwater pearl mussel populations known in the River Iijoki catchment is now 29, with a combined population of about 300,000 individual mussels (Table 4). Before the project, 20 freshwater pearl mussel populations were known to exist in this catchment. The estimated number of mussels varied from 1 (River Välijoki) to 50,000 (River Lohijoki), with 11 populations estimated to contain at least 10,000 mussels (Table 4).

6 Discussion

6.1 Suitability of electrofishing method in finding pearl mussel populations

Electrofishing and examination of glochidiosis at the site may provide a useful method to search for unknown freshwater pearl mussel populations, as well as to estimate the number of glochidia in fish. The present results show that glochidiosis can be accurately observed with a quick dip of fish gills by the naked eye at the site of collection, which would provide a reliable, non-destructive method to search for reproductive freshwater pearl mussel populations, as the fish can be released.

However, there are certain restrictions that have to be taken into account when applying the electro-fishing method. (1) Seasonally, the applicability of the method is limited to spring and early summer, as later in the summer the glochidia will detach from the host fish, and in the autumn the newly attached glochidia cannot reliably be seen due to their small size. (2) The electrofishing method is suitable for finding populations with only moderately intensive glochidia production, as the method is reliable only when the number of glochidia per fish is more than 20. (3) The method also is dependent on finding host fish, young salmonids, which may be challenging in spring. (4) Carrying electro-fishing gear is demanding and electrofishing requires at least a two-person team. (5) It is not known how far the glochidia drift in

Table 4. Known *M.margaritifera* populations from the River Iijoki water system after the present project (a total of 29 populations), with estimated numbers of mussels, and occurrence of small (< 4 cm) individuals.

N:o	Tributary	Sub-Drainage	Municipality	Pop.size	<4 cm mussels
1	Portinjoki	Harjajoki	Taivalkoski	10,000	observed
2	Kalajoki	Kalajoki	Taivalkoski	200	not observed
3	Susioja	Kostonjoki	Taivalkoski	100	not observed
4	Juurikkaoja	Juurikkaoja	Taivalkoski	500	not observed
5	Kostonlammenoja	Ohtaoja	Taivalkoski	10	not observed
6	Majovanoja	Majovanoja	Taivalkoski	50	not observed
7	Porraslammenoja	Majovanoja	Taivalkoski	1,000	observed
8	Pahkaoja	Majovanoja	Taivalkoski	200	not observed
9	Koivuoja	Koivuoja	Taivalkoski	3,000	observed
10	Alahaapuanoja	Haapuanoja-Virsuoja	Pudasjärvi	1,500	observed
11	Haukioja	Haukioja	Pudasjärvi	16,600	observed
12	Lohijoki	Lohijoki	Suomussalmi, Taivalkoski, Pudasjärvi	50,000	observed
13	Hukkajoki	Hukkajoki-Tervajoki	Suomussalmi	30,000	observed
14	Jukuanoja	Kouvanjoki lower part	Pudasjärvi	1,000	not observed
15	Latva-Kouvanoja	Kouvanjärvi	Pudasjärvi	< 10	not observed
16	Välijoki	Isojärvi	Taivalkoski	< 10	not observed
17	Korvuanjoki	Korvuanjoki middle part	Taivalkoski	500	not observed
18	Livojoki	Livojärvi	Pudasjärvi	8,500	not observed
19	Kouvanjoki	Kouvanjoki	Pudasjärvi	?	?
20	Kisosjoki	Kisosjoki	Taivalkoski	100	not observed
21	Laivajoki	Laivajoki	Posio	500	not observed
22	Norssipuro	Lukkarinoja	Pudasjärvi	20,200	observed
23	Nuottioja	Nuottijoki	Puolanka	40,000	observed
24	Myllypuro	Nuottijoki	Puolanka	25,000	observed
25	Ruokopuro	Suolijärvi	Puolanka	12,500	not observed
26	Tervajoki	Tervajoki	Puolanka	35,000	not observed
27	Vääräjoki	Särkijoki-Kinkelinjoki	Puolanka	20	not observed
28	Näätäjoki	Näätäjoki	Puolanka	30,000	not observed
29	Kokko-oja	Askanjoki lower part	Pudasjärvi	100	not observed

the water; if glochidia-infested fish are found, the exact location of freshwater pearl mussels have to be found with traditional methods. In the present project, the traditional method proved to be very effective – six new populations were found in practically one day, even though the rivers were of course not randomly chosen, but the most promising sites were selected for searching.

In spite of these restrictions, three new, previously unknown pearl mussel populations were found in the present project with the new electrofishing method from the River Iijoki catchment. In conclusion, although the electrofishing method cannot replace the traditional methods, it may provide a quick, inexpensive way of checking the occurrence of pearl mussels in a river. In some cases, the electrofishing method also makes it possible to observe rare/patchy mussel beds that would be very difficult to find using traditional mussel survey methods, for example in a very long waterway. A positive point is also that a good view of the salmonid population, or the entire fish community, may be achieved in addition to the occurrence of freshwater pearl mussel – including e.g. the growth of trout and salmon populations in the area of research as well as that of other species of fish,

such as the nine-spined stickleback (*Pungitus pungitus*) in the water systems.

6.2 Search for new freshwater pearl mussel populations

The vast areas investigated for the freshwater pearl mussels in the River Näätämö and River Teno water systems, both by the electrofishing method and by diving resulted in zero findings – no signs of freshwater pearl mussel. The waters in the area under observation are primarily fairly sensitive to acidification. Consequently, places can be subjected to peaks of acidity, which can cause disturbances in the reproduction of freshwater pearl mussels (see Taskinen et al. 2011). This characteristic of the waters of the area can be a partial reason why the populations of the freshwater pearl mussels were not found.

The search of the River Iijoki water system revealed nine new pearl mussel populations during the present project, so that the total number of freshwater pearl mussel populations of the River Iijoki drainage is now 29. This is a significant improvement in the knowledge of freshwater pearl mussel distribution and occurrence in the River Iijoki area, and further emphasizes the value of the River Iijoki catchment nationally and internationally for the conservation of the freshwater pearl mussel. In addition, one third of the populations contain young mussel individuals which makes a remarkable contribution to the number of vital freshwater pearl mussel populations in Finland. On the other hand, the result also shows that in the River Iijoki drainage only a fraction of the pearl mussel populations are such that their recruitment status is good.

Appendix 1

Locations of electrofishing surveys performed in the River Näätämö and Rived Teno water systems (plus one River Paatsjoki drainage site). Occurrence of brown trout/salmon, occurrence of < 100 mm brown trout/salmon and occurrence of other fish species are also given.

ID/N: o	River	Catchment	Finnish discharge code	Coordinates Lon-Lat	Trout/ Salmon	< 100 mm trout/salmon	OBS / NB
46	Hâcâstâmjuuha	Näätämö	69.032	27.6824 69.4230			minnow, nine-spined stickelback
45	Paavalijoki	Näätämö	69.033	27.9306 69.5113			minnow, nine-spined stickelback
39	Näätämöjoki	Näätämö	69.024	28.0963 69.5187	●		trout, burbot, minnow
42–44	Vaijoki	Näätämö	69.041	27.7927 69.5715 27.7790 69.5588 27.6806 69.4693	●		trout, grayling, pike, minnow, burbot
41	Čoarvejohka	Näätämö	69.044	27.6233 69.4522			
40	Nikolasjoki	Näätämö	69.051	27.5881 69.4400			minnow
38	Avlijuuha	Näätämö	69.025	28.2531 69.5224			grayling, pike, minnow, burbot
37	Ahvenjärvenjoki	Näätämö	69.069	28.4902 69.5235			minnow
36	Aili-joki	Näätämö	69.069	28.5934 69.5172	●	●	trout, minnow
35	Nuorttijoki	Näätämö	69.013	29.1211 69.6789	●	●	trout
21	Joki /Ukonselkä–Jänisjärvi	Näätämö	69.061	28.7096 69.5817	●	●	trout
34	Rautujoki	Näätämö	69.062	28.6426 69.5525			minnow
33	Juovssajärvi–Sevettijärvi	Näätämö	69.062	28.6249 69.5358			minnow
32	Kotajärvi–Teppana Kotajärvi	Näätämö	69.063	28.4408 69.4196	●		trout, minnow
31	Vaassalijärvi–Kotajärvi	Näätämö	69.063	28.4478 69.4201	●		trout, minnow
30	Hanhivuotso	Näätämö	69.066	28.6128 69.4688	●		trout, burbot, minnow, pike (soars on the pike)
29	Čoolmâsjuuha	Näätämö	69.066	28.6201 69.4756	●		trout
28	Nilijoki	Näätämö	69.063	28.5551 69.4888	●	●	trout
27	Rautaperänjoki	Näätämö	69.063	28.5222 69.4748	●		trout (bright), burbot, minnow
26	Petsijoki	Näätämö	69.068	28.5342 69.4938	●		trout, burbot, minnow
25	Unhorjuuha	Näätämö	69.067	28.2985 69.3769			
24	Harrioja	Näätämö	69.063	28.3699 69.4190			
23	Rautujoki	Teno	68.083	27.1894 69.5098			grayling
22	Moalkejohka	Teno	68.083	27.2221 69.5219	●	●	trout
20	Nameless stream to Lake Sollumusjärvi	Näätämö	69.066	28.7476 69.4932			burbot, minnow
19	Kurttajävri–Sollomusjärvi	Näätämö	69.066	28.7587 69.4882			minnow, grayling
7	Pätsikotajävri–Kurttejävri	Näätämö	69.066	28.8103 69.5003			nine-spined stickelback, minnow, burbot, pike

ID/N: o	River	Catchment	Finnish discharge code	Coordinates Lon-Lat	Trout/ Salmon	< 100 mm trout/salmon	OBS / NB
6	Nameless stream to Lake Vainosjärvi	Näätämö	69.065	28.8586 69.5068	●		trout, pike, minnow
5	Taimenlampi-Vainosjärvi	Näätämö	69.065	28.8883 69.5069	●		trout
4	Vainosjoki	Näätämö	69.065	28.9257 69.5475	●	●	trout, burbot
3	Pahtalampi–Siikajärvet	Näätämö	69.062	28.8455 69.5615			minnow, pike
2	Siikajärvet–Sanilanlampi	Näätämö	69.062	28.8385 69.5731	●		trout, grayling, minnow
1	Rautujoki	Teno	68.083	27.2185 69.4888	●	●	trout, burbot
0 8-12	Kietsimäjoki	Teno	68.04	25.1164 68.6367 25.1135 68.6677 25.1411 68.7789 25.1411 68.7789 25.1981 68.8108 25.2180 68.8197	●	●	trout, salmon, perch, pike, burbot, minnow
13	Utsjoki	Teno	68.082	27.2307 69.5822	●	●	salmon (inflamed gills)
14	Näätämöjoki (Kontinpaistama)	Näätämö	69.021	28.6184 69.6155	●	●	salmon (inflamed gills)
15	Gálddašjohka	Teno	68.057	27.9269 69.9783	●	●	salmon (inflamed gills)
16	Basijohka	Teno	68.03	26.0311 69.3945	●	●	trout
17	Luomusjoki	Teno	69.035	26.1386 69.3949			trout
18	Gákcavárjohka	Paatsjoki	71.984	26.3884 69.3515	●		trout
49	Suopumaoja	Näätämö	69.012	28.8984 69.7117	●	●	trout
50	Kallojoki	Näätämö	69.016	28.958 69.712	●	●	salmon
48	Harrijoki	Näätämö	69.014	28.7507 69.6889	●	●	salmon
47	Raanujoki	Näätämö	69.015	28.7442 69.6719	●		trout

Appendix 2

Locations of diving inventories performed in the River Näätämö and Rived Teno water systems. Municipality (M; U = Utsjoki, I = Inari), coordinates, length of transects and observations of fish are also given.

ID / N:O	River	Catchment	Finnish discharge code	M	Coordinates Start Lon-Lat	Coordinates End Lon-Lat	Length (m)	OBS/NB
1	River flowing from Vuolimuš Čiescadasjávri	Teno	68.087	U	26.9247 69.4428	26.9241 69.4429	30	minnow
3	Sávzajohka	Teno	68.088	U	26.9225 69.4424	26.9232 69.4429	45	trout, grayling, pike, perch, minnow
5	Sávzajohka	Teno	68.088	U	26.9218 69.4426	26.9229 69.4426	80	minnow, pike
7	River flowing from Lake Keräsjärvi (Cuoggá)	Teno	68.087	U	26.9201 694421	26.9208 69.4421	30	pike, perch, minnow
9	Cuoggá	Teno	68.086	U	27.0268 69.5183	27.0303 69.5189	150	trout
11	Vuokkujoki	Teno	68.089	U	26.9653 695815	26.9674 69.5814	100	trout, grayling
13	Kevojoki	Teno	68.091	U	26.9454 69.6744	26.9470 69.6743	80	trout/salmon, grayling
15	Kuksajoki	Teno	68.085	U	27.2372 69.5794	27.2383 69.5788	100	trout/salmon, minnow, grayling
17	Iivanajoki	Teno	68.041	I	25.7295 68.9808	25.7294 68.9813	50	grayling
19	Váddejohka	Teno	68.041	I	25.7592 68.9978	25.7512 68.9961	570	trout, grayling
21	Suolusjoki	Teno	68.032	I	25.7438 69.1336	25.7418 69.1338	100	salmon
23	Guottoveaijohka	Teno	68.038	I	25.7470 69.1347	25.7463 69.1349	40	salmon, trout
25	Leaibejohka	Teno	68.081	U	27.1199 69.6725	27.1120 69.6722	370	trout
28	Hárátjohka	Teno	68.082	U	27.2013 69.6389	27.1993 69.6379	130	trout
30	Ivvánasjohka	Teno	68.082	U	27.2839 69.6318	27.2827 69.6307	150	
32	Utsjoki river (Ollila)	Teno	68.082	U	27.0851 69.6353	27.0846 69.6391	450	too deep, salmon, grayling
	Utsjoki river (Mieraskoski)	Teno	68.083	U			40	Fresh Water Pearl Mussel
34	Tsieskuljoki	Teno	68.081	U	27.0737 69.7378	27.0703 69.7384	160	trout
36	Duolbajohka	Teno	68.062	U	27.3878 69.7956	27.3867 69.7950	100	grayling, minnow
38	Vetsijoki	Teno	68.062	U	27.3805 69.7962	27.3798 69.7990	330	grayling
40	Sorrája	Teno	68.062	U	27.3905 69.8133	27.3891 69.8127	100	grayling
42	Háltejohka	Teno	68.062	U	27.4358 69.8494	27.4345 69.8490	80	grayling
44	Bávvalašjohka	Teno	68.066	U	27.5908 69.9053	27.5785 69.9046	500	trout
46	Bávvalašjohka	Teno	68.066	U	27.5591 69.9058	27.5538 69.9073	270	trout, pike
48	Vuokŋoljohka	Teno	68.066	U	27.4572 69.9069	27.4570 69.9077	100	grayling
51	Báritjohka	Teno	68.066	U	27.4576 69.9086	27.4572 699083	40	
52	Skáidejohka	Teno	68.051	U	27.9542 69.9438	27.9549 69.9446	100	trout
54	Bajit Boratbokcájohka	Teno	68.015	U	27.6603 70.0203	27.6620 70.0211	150	trout
56	Vuolit Boratbokcájohka	Teno	68.012	U	27.7263 70.0264	27.7285 70.0266	150	trout
58	Utsjoki river (Upstream from Lake Mierasjärvi)	Teno	68.084	U	27.1427 69.4933	27.1468 69.4949	300	minnow, pike

Appendix 3

Rivers surveyed by electrofishing in the River Iijoki catchment in 2011–2013.

N:o	Tributary	Sub-Drainage	Municipality
1	Kylmäjoki yp	Näljänkäjoki	Suomussalmi
2	Kylmäjoki ap	Näljänkäjoki	Suomussalmi
3	Porraslammenoja	Majovanoja	Taivalkoski
4	Majovanoja ap	Majovanoja	Taivalkoski
5	Latvajoki	Korvuanjoki	Taivalkoski
6	Lahnasenoja	Korvuanjoki	Taivalkoski
7	Loukusanjoki	Iijoki	Taivalkoski
8	Pirinoja	Iijoki	Taivalkoski
9	Tutuoja	Iijoki	Taivalkoski
10	Pahkaoja	Iijoki	Pudasjärvi
11	Kisosjoki middle	Kisosjoki	Taivalkoski
12	Karhuoja	Kisosjoki	Taivalkoski
13	Kisosjoki ap	Kisosjoki	Taivalkoski
14	Kisosjoki yp	Kisosjoki	Taivalkoski
15	Paljakkaoja	Korvuanjoki	Puolanka
16	Lukkarinoja	Korpijoki	Pudasjärvi
17	Askanjoki	Korpijoki	Pudasjärvi
18	Tervaoja	Iijoki	Taivalkoski
19	Majovanoja yp	Majovanoja	Taivalkoski
20	Jaaskamonoja yp	Livojoki	Pudasjärvi
21	Jaaskamonoja ap	Livojoki	Pudasjärvi
22	Aimojoki yp	Mäntyjoki	Posio
23	Aimojoki ap	Mäntyjoki	Posio
24	Mäntyjoki	Livojoki	Posio
25	Majovanoja	Oudonjoki	Taivalkoski
26	Oudonjoki	Oudonjoki	Taivalkoski
27	Riitainjoki	Iijoki	Taivalkoski
28	Koiraoja	Kostonjoki	Taivalkoski
29	Latvajoki	Loukusanjoki	Taivalkoski
30	Visaoja	Loukusanjoki	Taivalkoski
31	Kutinjoki	Kostonjoki	Taivalkoski
32	Rääpysoja	Kostonjoki	Taivalkoski
33	Ahmaoja ap	Kostonjoki	Taivalkoski
34	Ahmaoja yp	Kostonjoki	Taivalkoski
35	Siiranjoki	Kostonjoki	Taivalkoski
36	Elehvänoja	Kostonjoki	Taivalkoski
37	Pahkaoja ap	Majovanoja	Taivalkoski
38	Pahkaoja yp	Majovanoja	Taivalkoski
39	Kostonlammenoja ap	Ohtaoja	Taivalkoski
40	Ohtaoja yp	Ohtaoja	Taivalkoski
41	Porraslammenoja	Majovanoja	Taivalkoski
42	Kylmävaaranpuro	Majovanoja	Taivalkoski
43	Hietajoki	Iijoki	Kuusamo
44	Hietajoki	Iijoki	Kuusamo
45	Martinjoki	Iijoki	Kuusamo

N:o	Tributary	Sub-Drainage	Municipality
46	Kylmäluomanoja	Iijoki	Taivalkoski
47	Ylähaapuanoja yp	Iijoki	Pudasjärvi
48	Ylähaapuanoja ap	Iijoki	Pudasjärvi
49	Harjajoki yp	Kostonjoki	Taivalkoski
50	Harjajoki ap	Kostonjoki	Taivalkoski
51	Kostonlammenoja yp	Ohtaoja	Taivalkoski
52	Ohtaoja ap	Ohtaoja	Taivalkoski
53	Tolpanoja	Puhosjoki	Pudasjärvi
54	Puhosjoki ap	Puhosjoki	Pudasjärvi
55	Puhosjoki yp	Puhosjoki	Pudasjärvi
56	Lohioja	Korpijoki	Pudasjärvi
57	Askanjoki	Korpijoki	Pudasjärvi
58	Väätäjänoja	Korpijoki	Pudasjärvi
59	Loukusanjoki	Iijoki	Taivalkoski
60	Kutinjoki yp	Kostonjoki	Taivalkoski
61	Siiranjoki yp	Kostonjoki	Taivalkoski
62	Siiranjoki ap	Kostonjoki	Taivalkoski
63	Kutinjoki ap	Kostonjoki	Taivalkoski
64	Vääränoja	Korvuanjoki	Taivalkoski
65	Korvuanjoki	Korvuanjoki	Taivalkoski
66	Välijoki	Isojärvi	Taivalkoski
67	Ruokosenpuro	Suolijärvi	Puolanka
68	Vääräjoki	Kinkelinjoki	Puolanka
69	Tervajoki	Tervajoki	Puolanka
70	Juurikaisenpuro	Kinkelinjoki	Puolanka
71	Lukkarinjoki	Askanjoki	Puolanka
72	Myllyjoki	Näljänkäjoki	Puolanka
73	Nuottijoki yp	Näljänkäjoki	Puolanka
74	Nuottijoki ap	Näljänkäjoki	Puolanka
75	Iijoki, Hepokangas 1	Iijoki	Taivalkoski
76	Iijoki, Hepokangas 2	Iijoki	Taivalkoski
77	Iijoki, Hepokangas 3	Iijoki	Taivalkoski
78	Iijoki, Hepokangas 4	Iijoki	Taivalkoski

Appendix 4

Rivers surveyed by aquasope, snorkelling or SCUBA diving in the River Iijoki catchment in 2011–2013.

N:o	Trributary	Sub-Drainage	Municipality
1	Martinjoki	Iijoki	Kuusamo
2	Hietajoki	Iijoki	Kuusamo
3	Riitainjoki	Iijoki	Taivalkoski
4	Tervaoja	Iijoki	Taivalkoski
5	Kisosjoki	Iijoki	Taivalkoski
6	Pahkaoja	Iijoki	Taivalkoski
7	Jaaskamonoja	Livojoki	Pudasjärvi
8	Askanjoki	Korpijoki	Pudasjärvi
9	Lukkarinoja (+Norssipuro)	Korpijoki	Pudasjärvi
10	Lukkarinjoki	Askanjoki	Puolanka
11	Kylmäjoki	Näljänkäjoki	Suomussalmi
12	Ohtaoja	Iijoki	Taivalkoski
13	Aimojoki	Mäntyjoki	Posio
14	Siiranjoki	Kostonjoki	Taivalkoski
15	Rääpysoja	Kostonjoki	Taivalkoski
16	Pirinoja	Iijoki	Taivalkoski
17	Tutuoja	Iijoki	Taivalkoski
18	Nuottijoki	Näljänkäjoki	Puolanka
19	Myllypuro	Näljänkäjoki	Puolanka
20	Tolpanoja	Puhosjoki	Pudasjärvi
21	Ruokosenpuro	Suolijärvi	Puolanka
22	Vääräjoki	Kinkelinjoki	Puolanka
23	Tervajoki	Tervajoki	Puolanka
24	Välijoki	Isojärvi	Taivalkoski
25	Ahvenjoki	Kalliojoki	Suomussalmi
26	Pärjänjoki	Pärjänjoki	Taivalkoski
27	Rekipuro	Näljänkäjärvi	Suomussalmi
28	Liimakaisenpuro	Kurjenjoki	Kuusamo
29	Lakioja	Lukkarinoja	Pudasjärvi
30	Koronoja	Kostonjoki	Taivalkoski
31	Kirppupuro	Irnijärvi	Kuusamo
32	Heinioja	Suolijärvi	Suomussalmi
33	Hirvipuro	Näätäjoki	Puolanka
34	Tonko-oja	Askanjoki	Puolanka
35	Hepo-oja	Korpijoki	Puolanka
36	Junnonjoki	Kalliojoki	Suomussalmi
37	Laivajoki	laivajoki	Posio
38	Kokko-oja	Askanjoki	Pudasjärvi
39	Näätäjoki	Näätäjoki	Puolanka
40	Seimioja	Korvuanjoki	Taivalkoski

Annex G

Dissemination of information

List of the media activities and other occasions, where the information about the project and freshwater pearl mussel has been delivered is shown below in Tables 1–6.

Table 1. Oral presentations

Occasion	Place	Time
Nordic mussel workshop: Stormusslor	Storåbränna, Jämtland, Sweden	28–30.6.2011
Media briefing	Taivalkoski, Finland	12.9.2011
Seminar, freshwater pearl mussel	Pudasjärvi, Finland	19.11.2011
Seminar, River restorations	Jyväskylä, Finland	30.11.2011
Briefing for media, authorities and local people	Paltamo, Finland	8.2.2012
Informative meeting in Kainuu ELY-Centre	Kajaani, Finland	20.2.2012
Steering group meeting	Rovaniemi, Finland	13.3.2012
Project workshop	Svanvik, Norway	28–29.3.2012
Briefing for media, authorities and local people	Inari, Finland	3.5.2012
Briefing for media, authorities and local people	Karasjok, Norway	4.5.2012
7th International Acid Sulphate Soil Conference	Vaasa, Finland	26–31.8.2012
Doctoral Program in Integrated Catchment and Water Resources Management (VALUE) – graduate school seminar	Tvärminne, Finland	2–3.10.2012
International meeting on biology and conservation of freshwater bivalves	Braganga, Portugal	4–7.9.2012
Project partners workshop	Pudasjärvi, Finland	January 2013
Workshop with project "Kainuu pearl fishers"	Pudasjärvi, Finland	February 2013
Workshop between the cross-border projects	Rovaniemi, Finland	February 2013
International seminar on "Practical Implementation of FPM Measures"	Letterkenny, Ireland	15.2.2013
Steering group meeting	Rovaniemi, Finland	March 2013
International mussel congress	USA	March 2013
Partner meeting	Konnevesi, Finland	April 2013
Briefing for the forestry operators	Oulu, Finland	April 2013
Briefing for media, authorities and local people	Pudasjärvi, Finland	May 2013
Briefing for the forestry operators	Rovaniemi, Finland	June 2013
16th International Conference on Diseases of Fish and Shellfish	Tampere, Finland	September 2013
International meeting on "Improving the Environment for the Freshwater Pearl Mussel"	Kefermarkt, Austria	13–15.11.2013
Steering group meeting	Rovaniemi, Finland	12.5.2014
International meeting on "Raakku! – Restoration of the Freshwater Pearl Mussel Populations with New Methods" (project's final seminar)	Rovaniemi, Finland	13–15.5.2014
Mussel workshop	Ytterhogdal, Jämtland, Sweden	3–5.6.2014

Table 2. Poster presentations

Poster presentations	Place	Time
International Meeting of Biology and Conservation of Freshwater Bivalves	Braganca, Portugal	September 2012
International Conference on Diseases of Fish and Shellfish	Tampere, Finland	September 2013
TRIWA III- Interreg IVA -project´s end seminar	Övertorneå, Sweden	May 2014
International meeting on "Raakku! – Restoration of the Freshwater Pearl Mussel Populations with New Methods" (project's final seminar)	Rovaniemi, Finland	May 2014

Table 3. Scientific articles

Authors and Title	Journal
J. Taskinen, P. Berg , M. Saarinen-Valta , S. Välilä , E.Mäenpää , K. Myllynen & J. Pakkala 2011. "Effect of pH, iron and aluminum on survival of early life history stages of the endangered freshwater pearl mussel, Margaritifera margaritifera". http://www.ingentaconnect.com/content/tandf/gtec/2011/00000093/00000009/art00006	Toxicological & Environmental Chemistry.

Table 4. Newspaper and journal articles.

Title (articles in Finnish)	Newspaper/Journal	Time
"Isäntäkalatutkimus raakkupurossa Taivalkoskella"	Koillissanomat	14.9.2011
"Lohet raakulle elinehto"	Kaleva	15.9.2011
"Jokihelmisimpukkakantoja elvytetään uusin menetelmin"	Koillismaan uutiset	15.9.2011
"Raakun lisääntymistä tutkitaan Iijoen vesistössä"	Iijokiseutu	16.9.2011
"Raakun pelastajilla on kiire".	Maaseudun tulevaisuus	19.10.2011
"Kello käy jokihelmisimpukalle"	Lapin Kansa	4.5.2012
"Kello käy jokihelmisimpukalle"	Pohjolan Sanomat	4.5.2012
"Raakut mittaavat purojen kunnon"	Lapin Kansa	7.5.2012
"Jokihelmisimpukka kaipaa kaverikseen lohen tai taimenen"	Metsä.fi	June 2012
"Katoavat helmet"	Apu 34/12	23.8.2012
"Jokihelmisimpukka kaipaa kaverikseen lohen tai taimenen"	Veto-Uistelu 3/12	March 2012
"Aigot ealaskahttit johkakalzzu" (In North Sami)	Avvir	5.5.2012
News from the briefing in Pudasjärvi 8.5.2012	Kaleva	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Kaleva	9.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Keskipohjanmaa	9.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Kouvolan Sanomat	9.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Etelä-Saimaa	9.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Itä-Savo	9.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Pohjolan Sanomat	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Ilkka	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Pohjalainen	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Aamulehti	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Savon Sanomat	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Lapin Kansa	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Kainuun Sanomat	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Turun Sanomat	8.5.2013
News from the briefing in Pudasjärvi 8.5.2012	Keski-Pohjanmaa	8.5.2013
"Älä tallaa raakkuja"	Erä 7/13	July 2013
"Raakku kaipaa rauhaa"	Suomen kuvalehti	27.9.2013
"Metsähallitus: Suomen raakkukannan tila on hälyttävä"	Turun Sanomat	13.5.2014
"Raakku vaarassa kadota Suomesta – tila hälyttävä"	Iltalehti	13.5.2014
"Vedenpudistaja raakku vähenee- Arkitukumin kokous esittelee uhanalaisen lajin"	Lapin Kansa	13.5.2014
"Metsähallitus: Suomen raakkukannan tila on hälyttävä"	Kainuun sanomat	13.5.2014
"Raakkujen tila paljastui pelättyä huonommaksi"	Vihreä lanka	13.5.2014
"Uhanalaisen raakun tila hälyttävä"	YLE Uutiset	13.5.2014
"Jokihelmisimpukat vaarassa hävitä Suomen joista"	Maaseudun tulevaisuus	13.5.2014
"Metsähallitus: Suomen raakkukannan tila on hälyttävä"	Pohjolan sanomat	13.5.2014
"Metsähallitus: Suomen raakkukannan tila on hälyttävä"	Länsi-Suomi	13.5.2014
"Metsähallitus: Suomen raakkukannan tila on hälyttävä"	Aamulehti	13.5.2014
"Raakun elinympäristöt aiotaan suojella"	Koillissanomat	14.5.2014
"Raakkukannan tila on hälyttävä"	Demokraatti	14.5.2014
"Raakun elinympäristöt aiotaan suojella"	Kymen sanomat	14.5.2014
"Jokihelmisimpukka lisääntyy enää parissa purossa"	Kaleva	14.5.2014
"Raakun elinympäristöt aiotaan suojella"	Savon sanomat	14.5.2014
"Raakkukannan tila on hälyttävä Suomessa"	Keskipohjanmaa	14.5.2014
"Jokihelmisimpukan elinympäristöt suojeluun"	Satakunnan Kansa	17.5.2014
"Raakut kertovat purojen kunnosta"	Lapin Kansa	15.5.2014
"Raakku on kranttu"	Lapin kansa	17.5.2014
"Rakas raakku riutuu"	Suomen luonto	June 2014

Table 5. Radio and TV programs

Program	Media	Time
Huomenta-Saami	YLE Sami radio	6.9.2011
"Jokihelmisimpukan pelastusoperaatio käynnisty""	YLE Pohjois-Pohjanmaa	13.9.2011
"Raakkututkijat kaipaavat perinnetietoa raakusta"	YLE Sami radio	4.11.2011
Radio news	Radio Suomi/ Oulun alueradion	20.11.2011
Johkaskálžžut leat áitojuvvon davviguovlluid jogain	YLE Sami radio	3.5.2012
Luonto lähellä	YLE TV 1	October 2013
Puoli seitsemän	YLE TV 1	7.1.2014

Table 6. Social media

Media	Address
Project's web page	www.metsa.fi/sivustot/metsa/fi/Hankkeet/Rakennerahastohankkeet/jokihelmisimpukka/Sivut/default.aspx. Language versions: Finnish, Swedish, North Sami, Skolt Sami, Anar Sami
Species of the month campaign: Youtube video 2 blog posts Poster	www.metsa.fi/rakkaudestalajiin

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