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Electrofishing as a new method to search for unknown populations of the endangered freshwater pearl mussel *Margaritifera margaritifera*

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Running Head: Electrofishing to search for *M. margaritifera*

Keywords: brown trout; distribution; electrofishing; endangered species; freshwater pearl mussel; glochidia; occurrence; river

Abstract

1. The freshwater pearl mussel *Margaritifera margaritifera* is threatened throughout its Holarctic range, but the occurrence of this species is insufficiently mapped. For the conservation of *M. margaritifera*, it is important to identify populations more comprehensively.
2. Traditionally mussels have been searched for visually using techniques such as diving and aquascope, both of which are potentially time-consuming and demanding survey methods.
3. In this study, a new search method is presented. As glochidia of *M. margaritifera* are larval parasites on gills of salmonid fish, electrofishing and non-destructive examination of salmonids with the naked eye may reveal the presence of glochidia and therefore the occurrence of *M. margaritifera* in watercourses. This method was tested in both the field and laboratory in northern Finland.
4. In summer, when *M. margaritifera* glochidia were large, the status of salmonids being infected or uninfected by *M. margaritifera* was correctly identified with the naked

eye with 62, 80, 88 and 93 % accuracy in four streams sampled, 96 % accuracy in the laboratory, and 100 % accuracy in all cases when at least 20 glochidia per fish were present. Intensity of infection was also assessed successfully; a specifically tailored, qualitative abundance score correlated significantly with the real number of glochidia. However, during autumn with small glochidia freshly attached to fish, glochidia infection could be observed only under microscopic examination.
5. When the method was used in 40 previously incompletely surveyed tributaries, three *M. margaritifera* populations were found. The infection in salmonids was observed always with the naked eye, being subsequently confirmed microscopically. The existence of adult mussels in two of these rivers was also confirmed.
6. The results indicate that electrofishing and a relatively quick naked-eye check of salmonids provides a new, non-destructive, and potentially cost-effective way to search for new, previously unrecorded *M. margaritifera* populations.

INTRODUCTION

The freshwater pearl mussel, *Margaritifera margaritifera*, is a threatened bivalve mollusc which lives in pristine running waters in the Holarctic region and is often considered as an indicator of natural, undisturbed habitats (Geist, 2010). However, the occurrence of *M. margaritifera* is not precisely known within its range, with 'new', previously unknown and even remarkably large or genetically important populations still being found occasionally (Álvarez-Claudio *et al.*, 2000; Reis, 2003; Ostrovsky and Popov, 2011; Oulasvirta, 2011; Varandas *et al.*, 2013). Therefore, although *M. margaritifera* populations from extensively studied river systems are probably well documented, a considerable number of unknown populations may still exist in insufficiently surveyed streams and rivers (Cosgrove *et al.*, 2000; Oulasvirta, 2011). In particular, the occurrence of *M. margaritifera* in remote areas of northernmost Europe has not been mapped thoroughly, and when information regarding its occurrence is available, the details are often sporadic, e.g. historical pearl fishing records or local, unconfirmed knowledge (Young and Williams, 1983b; Oulasvirta, 2011; Simon *et al.*, 2015). To avoid the accidental disturbance of not yet recorded populations via e.g. forestry operations, ditching, or peat mining – activities that are widespread and causes

of freshwater pearl mussel population extinctions especially in the north (Oulasvirta, 2011) – it is highly important to have detailed information on the distribution of *M. margaritifera*. In addition, there may also be a need to characterize the current status of *M. margaritifera* in rivers or river sections where the species has been reported in the past (Oulasvirta, 2011).

M. margaritifera has a complex life cycle (e.g. Young and Williams, 1984a; Bauer, 1987b; Hastie and Young, 2001; Geist *et al.*, 2006), which includes up to almost one year in a parasitic phase on gills of a salmonid host fish, Atlantic salmon (*Salmo salar*) or brown trout (*Salmo trutta*). This is followed by a post-parasitic stage in which the juvenile mussel is completely burrowed in the river substratum for several years. After these critical stages, adult *M. margaritifera* can reportedly live more than 200 years (Ziuganov *et al.*, 2000; Helama and Valovirta, 2008). Anthropogenic perturbations, including siltation, loss of habitats, pollution, loss of host fish, introduction of invasive species, and commercial exploitation have caused a substantial decline of freshwater mussels worldwide (Bauer, 1988; Williams *et al.*, 1993; Lydeard *et al.*, 2004). Many of these factors have been suggested to be the reasons for the decline of *M. margaritifera*, too, with the species now being extinct or close to extinction in many areas (Young and Williams, 1983b; Bauer, 1986, 1988; Cosgrove *et al.*, 2000; Oulasvirta, 2011; Simon *et al.*, 2015). At present, *M. margaritifera* is classified as critically endangered in Europe (Cuttelod *et al.*, 2011~~IUCN Red List of Threatened Species, 2013~~).

Today, mapping the occurrence of *M. margaritifera* consists of visual techniques like SCUBA diving, snorkeling, using an aquascope or underwater camera, or observing mussels visually from the shore or in a boat (Álvarez-Claudio *et al.*, 2000; Reis, 2003; Cosgrove *et al.*, 2000, 2007; Oulasvirta, 2011; Varandas *et al.*, 2013; Simon *et al.*, 2015). However, these methods can be time-consuming, and factors like dark or turbid water, boulder substrate, aggregated distribution of mussels, deep water or large areas of unsurveyed watercourses may limit their applicability (Young and Williams, 1983a; Álvarez-Claudio *et al.*, 2000; Cosgrove *et al.*, 2000; Strayer and Smith, 2003; Oulasvirta, 2011). Thus, alternative methods for determining the presence of mussels in unsurveyed watercourses would be welcome. Since *M. margaritifera* is dependent on a

host fish to complete its life cycle, one such method could be capturing potential host fish individuals and examining them for *M. margaritifera* glochidia. The glochidia are relatively small (ca. 70 µm) at the beginning of the parasitic phase in autumn, but when fully developed, they are much larger (400–500 µm) and possibly observable with the naked eye (e.g. Young and Williams, 1984b; Bauer and Vogel, 1987; Pekkarinen and Valovirta, 1996). Thus, it should be possible to detect the occurrence of *M. margaritifera* glochidia without killing the host fish for microscopic examination. Electrofishing is a widely used method to capture small salmonids non-destructively (Hudy, 1985; Bohlin *et al.*, 1989), and is not harmful to mussels (Hastie and Boon, 2001). It is, therefore, possible that *M. margaritifera* glochidia can be seen by quickly looking into the gills of electrically caught and stunned, and afterwards released, host salmonids. Overall, this method could serve as a new, non-destructive way to search for undescribed freshwater pearl mussel populations.

An equivalent method of identifying *M. margaritifera* glochidia in fish caught by electrofishing was earlier developed and presented by Österling (2011). In that study, the intensity of *M. margaritifera* infection was estimated by photographing gills of anaesthetised brown trout, with a metal spatula inserted between its gill arches. The results indicated that a reliable estimate of the number of glochidia can be determined by examining photographs of fish gills (Österling, 2011). In addition, the method, including electrofishing, anaesthetising, and photographing was non-destructive given that the survival and growth of brown trout was not affected by these treatments (Österling, 2011). In the present study, the procedure of Österling (2011) is modified to develop a new technique for uncovering natural populations of *M. margaritifera*. First, it was tested whether the occurrence and the intensity of *M. margaritifera* infection in potential fish hosts could be reliably estimated quickly with the naked eye immediately after fish are caught by electrofishing. Secondly, the method was used in practice to search for previously unknown *M. margaritifera* populations in northern Finland.

METHODS

Testing the method: finding *M. margaritifera* glochidia using the naked eye

The method was first tested in four small streams in the River Iijoki catchment (14200 km^2, draining to the Baltic Sea) in Finland, northern Europe (Figure 1). These rivers with average depth 0.22 to 0.63 m and width 1.1 to 4.6 m were previously confirmed to harbour freshwater pearl mussel. In 2011, the electrofishing was carried out close to the known mussel beds of the Rivers Jukuanoja (total river length 4.7 km) and Koivuoja (14.7 km) five times during summer to assess the compatibility of the method to *M. margaritifera* glochidia of different sizes. Information about the life cycle of freshwater pearl mussel from these insufficiently investigated populations was also collected. In 2012–2013, the study sites were the Rivers Majovanoja (13.5 km) and Pahkaoja (2.4 km) which were fished once in June, as the 2011 survey revealed that this is the optimal time to find salmonids infected by large *M. margaritifera* glochidia. The preliminary goal was to collect five juvenile brown trout or salmon individuals per river on each survey occasion, given that the young salmonids are the most suitable hosts for *M. margaritifera* (e.g. Young and Williams, 1984a; Hastie and Young, 2001; Österling and Wengström, 2015). However, because the salmonid populations in the rivers appeared not to be too numerous and the acquired licenses also restricted the number of fish to be sacrificed, the sample sizes were reduced (see Results).

The electrofishing was conducted using GeOmega FA4 or Paulsen FA4 device, and the total time spent at each site varied from 15 to 90 minutes. In 2011, each brown trout was quickly checked by one person and classified as being infected or uninfected by *M. margaritifera* using only the naked eye, through gently opening the operculum using wet rubber gloves so that the fish was not harmed and could have been quickly released. In 2012–2013, the intensity of *M. margaritifera* infection was assessed in the field by two or three independent observers. Each of them scored the intensity on each fish as 0, 1, 2, 3, 4 or 5, where 0 indicated no glochidia found, while 5 indicated a very high number of glochidia found (Figure 2). These procedures, taking only a few seconds, were conducted immediately after the fish was caught and still stunned by the electric shock. After these actions, each fish was killed and stored on ice. Later, the total length (mm) of each fish was recorded and the number of *M. margaritifera* glochidia in their gills was determined microscopically. The length (µm) of glochidia was determined by

randomly picking 10 individuals per fish and measuring them using an ocular scale on the microscope.

The naked-eye scoring technique was also tested in laboratory using artificially infected, farmed brown trout. The one year old fish were exposed to *M. margaritifera* originating from the River Livojoki (Figure 1) in September 2014 at Konnevesi Research Station (University of Jyväskylä). Glochidia were collected by placing several adult mussels temporarily in a bucket with 5 L of water (see Young and Williams, 1984a; Bauer, 1987a), allowing them to release their glochidia naturally. Then a total of 1.5 L of glochidial suspension microscopically estimated to have approximately 180000 larvae were added to the 163 L tank containing 100 brown trout from the Rautalampi (Konnevesi) strain. In July 2015, the fish were scored for the intensity of *M. margaritifera* infection by two independent observers as outlined above.

An association between the mean of the naked-eye scores and the number of glochidia confirmed microscopically was analysed statistically with Spearman correlation analysis using IBM SPSS Statistics (version 20.0.2, IBM Corporation, New York, United States). Prior to this analysis, possible differences in the naked-eye scores between different observers were checked with Friedman test. River-level mean naked-eye scores for intensity of *M. margaritifera* infection were also created by first calculating the fish-level mean of the scores of two or three observers, and then calculating the total mean of these individual means within each river.

Applying the method: searching for *M. margaritifera* populations using the electrofishing method

The method was applied in June 2012 and June 2013 to 40 previously unsurveyed or incompletely surveyed rivers in the River Iijoki drainage (Figure 1). These rivers, potentially harbouring *M. margaritifera*, were selected for study after consultations with local experts who, for example, informed us about possible pearl fishing history in the area. Nonetheless, even the presence of salmonid fish was unknown in many of the rivers before the current study. Eight of the rivers were electrofished in both years, and

in some rivers the fishing site was changed several times if salmonids were not found. The total time spent electrofishing per river varied from 30 minutes to 195 minutes, and the target was to catch five young salmonid per river. However, salmonids were not found in all rivers, while in some sites the fish density appeared to be high and catches were increased (see Results). After each salmonid catch, the intensity of *M. margaritifera* infection was quickly scored from 0 to 5 (Figure 2) by two or three observers as above. The infection status and number of glochidia infecting each fish was confirmed later in the laboratory, and if any *M. margaritifera* glochidia were found, the occurrence of adult mussels in the river was verified. Mean naked-eye score for each river was calculated as above, and mean intensity of infection for each river was calculated to represent the mean number of glochidia per infected fish.

RESULTS

Testing the method: finding *M. margaritifera* glochidia using the naked eye

In both the Rivers Jukuanoja and Koivuoja, *M. margaritifera* glochidia that had attached to fish in the previous year were found still on fish gills in June and July but not during August, indicating the excystment of glochidia had occurred between late July and early August (Table 1). In late August, a new glochidium generation was attached to fish in the River Jukuanoja, but not among fish in the River Koivuoja (Table 1).

In June and July, when glochidia were large (i.e. shortly before they had detached from their hosts) the status of brown trout as being infected or uninfected by *M. margaritifera* was correctly classified in 13 out of 14 (93 %) fish in the River Jukuanoja, and in 8 out of 13 (62 %) fish in the River Koivuoja (Table 1). No false positive records were obtained, and all the false negative assessments were among the fish carrying 1 to 19 glochidia. Thus, the naked-eye classification of fish was 100 % correct for both rivers when there were at least 20 glochidia per fish (Table 1). The mean length of glochidia on each fish varied from 247 to 456 µm in June-July (Table 1). However, when the next generation of glochidia had attached to fish in the River Jukuanoja at the end of August,

each fish caught was misclassified as uninfected with the naked eye, although all of them were infected, most of them heavily (Table 1). The mean length of these "new" glochidia was 74–81 µm.

The catch from the Rivers Majovanoja and Pahkaoja in June 2012 and 2013 included 12 brown trout, with most of them carrying *M. margaritifera* glochidia of 202–296 µm in mean size (Table 2). Using two or three observers for each fish, the infection status was correctly classified in 12 out of 15 (80 %) cases in the River Majovanoja, 15 out of 17 (88 %) cases in the River Pahkaoja, and in 100 % of cases when there were more than 4 glochidia per fish (Table 2). Moreover, the mean naked-eye scores correlated significantly ($r_s = 0.864$, $p < 0.001$, $n = 12$) with the numbers of glochidia confirmed microscopically (Table 2). There were no significant differences (Friedman test, $p = 0.472$) in scores between different observers (Table 2). Furthermore, for individual fish, the mean of the naked-eye score values greater than 0.50 consistently identified *M. margaritifera* infection (Table 2). However, a mean score higher than 0 but less than or equal to 0.50 indicated a probability for a false positive classification (Table 2). For each river, the mean scores were much higher than 0.50; 2.20 for the River Majovanoja and 1.12 for the River Pahkaoja (Table 2).

In the laboratory experiment with artificially infected brown trout, glochidia were found in 11 of 40 fish, having a mean length of 339 to 409 µm (Table 3). With two observers, the infection status was correctly classified in 77 out of 80 (96 %) cases and in 100 % of cases where fish was carrying more than one larva (Table 3). The mean scores correlated significantly ($r_s = 0.931$, $p < 0.001$, $n = 40$) with the numbers of glochidia counted microscopically (Table 3). There were no significant differences between the scores of individual observers (Friedman test, $p = 0.257$), and the mean of the scores greater than 0.50 identified the infected fish in each case (Table 3).

Applying the method: searching for *M. margaritifera* populations using the electrofishing method

Among the 40 rivers surveyed, brown trout were caught from 22 rivers, with a total catch of 141 individuals (Table 4). *M. margaritifera* infection was recorded on 10 fish in three rivers, and in each of the rivers the infection was observed also with the naked eye in the field (Table 4). Thus, the application of the new electrofishing method revealed three *M. margaritifera* populations from the Rivers Kisosjoki, Lukkarinoja and Kostonlammenoja (Figure 1, Table 4). The presence of adult mussels in the Rivers Kisosjoki and Lukkarinoja were verified later during summer 2012. In the River Kisosjoki the verification, using an aquascope and snorkel, took about six hours, and a total of 15 adult mussels were found 100 m to 1 km above the electrofishing site. In the River Lukkarinoja, 5000 mussels were found in two hours a few km above the fishing location. The River Kostonlammenoja had been previously known to harbour *M. margaritifera*, but the population was thought to have become extinct (fisheries planner Eero Moilanen, Metsähallitus, *pers. comm.*). The river was not surveyed conventionally after the electrofishing, but according to our results it still harbours *M. margaritifera*.

At the river level, no false negative classifications were obtained, and in 14 rivers all uninfected fish were classified correctly by each observer (Table 4). However, a false positive classification was obtained in five rivers (Table 4). In each of these cases, the total river-level naked-eye score was very low (0.07 to 0.40 on the 0 to 5 scale), and as earlier, any incorrect classification did not occur when the mean naked-eye score was higher than 0.5 (Table 4). However, among the revealed FPM rivers, the mean score was less than 0.5 in 2 of the 3 cases (Table 4).

Overall, by testing and applying the electrofishing method, 197 wild brown trout were examined microscopically for mussel infection, and only glochidia belonging to *M. margaritifera* were identified. The reason for the few false positive records remains unknown, but may be due to tumours or external debris present on the fish gills.

DISCUSSION

Electrofishing is the main standardized method for capturing small salmonids non-destructively in lotic systems (Hudy, 1985; Bohlin *et al.*, 1989). The present results

show that electrofishing also can be used to search for freshwater pearl mussel (*Margaritifera margaritifera*) populations, with moderate to high intensity of *M. margaritifera* infection being reliably seen quickly with the naked eye on the gills of stunned host fish. Results of this study also indicate that the quick naked-eye examination can provide a good estimate of the intensity of the infection, and thus give some indication of glochidial production capacity of the mussel population without disturbing the sensitive mussel bed itself, and without depleting the host fish population.

However, there are some limitations should be taken into account when applying the electrofishing method. First, the method requires electrofishing equipment and at least a two-person team. Second, the reliable applicability of the method is limited to the late part of the parasitic stage of *M. margaritifera*, as the results indicate that freshly attached glochidia of size only 70–80 µm may not be reliably observed with the naked eye (Table 1). Thus, knowledge about the glochidial excystment time in the study area may help one to employ the electrofishing method at the right time, i.e. when glochidia are as large as possible but not yet detached from the fish. Unfortunately, this knowledge is not usually available when investigating unsurveyed rivers in remote areas, as in this study. We can, nonetheless, estimate that for example in the northern Europe the ideal time to examine salmonids for *M. margaritifera* infection is between spring and midsummer. Finally, the method seems to be most reliable when the number of glochidia per fish is more than 20 (Tables 1–3), although it is likely that with more experience the number of incorrect scores among fish of low numbers of glochidia (less than 20) would eventually decrease. It is also worth mentioning that if the number of mussels in a selected river is low, if reproduction in the population is not successful, or if the population occurs far below or far above the electrofishing site, the occurrence of glochidia and thus the mussel population may remain undetected.

Possible migration of salmonid hosts may potentially confound the observations. In the River Lukkarinoja, five brown trout were caught, but only one of them was infected by *M. margaritifera* (Table 4), and the mussel population was found a few kilometres above the electrofishing site. In this instance, it is likely that the infected fish had migrated to the fishing site from the mussel area. However, in general, juvenile brown

trout – the most common host of *M. margaritifera* in many areas (Young and Williams, 1984a; Hastie and Young, 2001; Österling and Wengström, 2015) although several populations which use only salmon as their only host have also been reported (Karlsson *et al.*, 2014) – tend not to move very far (Harcup *et al.*, 1984; Olsson and Greenberg, 2004). Thus, one should particularly focus on catching juvenile salmonids when searching for *M. margaritifera* populations using the presented electrofishing method. It is also important to note that glochidia of *M. margaritifera* may move from the habitat of adult mussels, as well; it has been shown that unattached larvae can survive for at least 8 days under optimal conditions (Taskinen *et al.*, 2011), and drift at least 500 m in a river and still remain infective (Hastie and Young, 2001). However, because glochidia only can move in a downstream direction, selecting a fishing site from downstream sections of potential mussel habitats would have a better chance of revealing *M. margaritifera* glochidia on fish hosts.

The limitations of the electrofishing method listed above also apply to the photo-identification method developed by Österling (2011), but with the photo-method it is possible to observe also the small, freshly attached *M. margaritifera* glochidia (Österling, 2011). However, observing *M. margaritifera* glochidia using only the naked eye takes less time and requires less equipment in the field than the photo-method. Furthermore, there is no need to install the metal spatula between the gill arches as used by Österling (2011), making the electrofishing method less intrusive to the fish. Some signs of minor spinal injuries in fish after electrofishing have been reported (Dalbey *et al.*, 1996), but in the study of Österling (2011), no increased mortality among any of the treatment was observed. Also, for most electrofishing surveys, it is normal to anaesthetise fish and measure their length prior to release. If information pertaining to fish length is not needed for the glochidial examination, then the electrofishing method described in this study does not require anaesthetisation, given that the visual check takes only a few seconds and can be done immediately after the catch while the fish is still stunned. Consequently, the fish can be released instantly after the glochidial examination because there is no need for fish to recover from the anaesthetisation. Thus, the fish handling time in the current electrofishing method is lower than in typical electrofishing surveys.

Despite the aforementioned limitations and a few false naked-eye classifications, the electrofishing method proved to be a practical method of surveying for freshwater pearl mussel populations. Among 40 study rivers in the River Iijoki catchment, brown trout were caught from 22 of them, and fish were found to harbour *M. margaritifera* infection in 3 rivers (Table 4). The occurrence of adult mussels was confirmed in 2 of these rivers, with the third river reportedly harbouring *M. margaritifera* population in the past but thought to have become extinct what it obviously is not. Thus, three new, potentially reproducing *M. margaritifera* populations were revealed in just in the first test of the method. Therefore, results of this study suggest that if salmonids with *M. margaritifera* glochidia are found, and especially if the fish or river level mean score of the observers is more than 0.5 using the naked-eye scoring system of the present study, one can get an indication that there is a robust, glochidium-producing freshwater pearl mussel population in the river. In addition, because the important role of mussels coming from their filtering and burrowing activity is recognized (e.g. Howard and Cuffey, 2006; Vaughn et al., 2008; Geist, 2010), they are especially the most important river ecosystems of high conservation value to be potentially discovered with the electrofishing method. Furthermore, the electrofishing method provides knowledge on the occurrence and the density of salmonids and other fish species in the selected rivers – valuable information that cannot be collected with the traditional mussel search methods. The quick glochidial examination would also be useful to incorporate within electrofishing and salmonid research, especially if the work is conducted in remote unsurveyed rivers – conjoining of two surveys into one provides better value for the work.

Whether the electrofishing method (or any traditional technique) is used, one cannot rule out the possibility of *M. margaritifera* being present in the river. Instead, the strength of the presented method is to find unknown populations of *M. margaritifera* potentially cost-effectively; indeed, a catch and quick assessment of only one fish may give an indication about the occurrence of the mussel in a river. The results also suggest a high repeatability of the naked-eye scoring technique, given that any statistical differences between the scores of the different observers were not found. Also, given

that captured fish can be released after the quick examination, applying the presented electrofishing technique even to the smallest brooks with very low number of salmonids is not problem as the method does not threaten the fish populations – it is worth to note that fish were not released in this first application of the method to be able to test its reliability and effectiveness, and due to that the sample sizes in the present study had to keep low in the cases of wild fish.

No glochidia from mussels other than *M. margaritifera* were found in any of the fish captured during this study. It is, however, possible that other mussel species, like the duck mussel, *Anodonta anatina*, (Oulasvirta, 2011) can occasionally occur in the same river and thus simultaneously infect the same fish. However, when electrofishing is carried out between spring and midsummer, as suggested above, the probability of fish carrying *A. anatina* glochidia is negligible, given that *A. anatina* releases its larvae to water in early spring and the parasitic stage lasts only a few weeks (Jokela *et al.*, 1991; Taskinen *et al.*, 1997). The thick-shelled river mussel, *Unio crassus,* can also be found in rivers containing *M. margaritifera* (Zettler and Jueg, 2007; Zieritz *et al.*, 2012), but salmonids are not suitable hosts for this species (Taeubert *et al.*, 2012). Nonetheless, the electrofishing method could be useful in searching for other mussel species, too, especially ones which have relatively large glochidia, and in cases of simultaneous infections, the mussel species can be identified morphologically (Pekkarinen and Englund, 1995a, b; Pekkarinen and Valovirta, 1996) or using molecular techniques (Gerke and Tiedemann, 2001; Zieritz *et al.*, 2012).

The current study area, the River Iijoki catchment altogether includes 1400 streams, with some of these streams being relatively dark, turbid or brown colored, hiding the mussels effectively and making it difficult to uncover populations of *M. margaritifera* using traditional sampling techniques. Furthermore, this catchment is a typical example of Finnish river systems, which together cover thousands of small rivers and streams and thus, probably many undiscovered *M. margaritifera* populations. Therefore, especially in that kind of catchments the electrofishing method described in this study may provide an effective means for searching for *M. margaritifera*, thus revealing its status and contributing to its conservation efforts. Although the presence of adult

mussels must continue to be determined, probably with traditional methods, we propose that the electrofishing method can serve as a valuable instrument for finding undiscovered populations of freshwater pearl mussel – possibly as a first line exploratory tool in large unmapped drainages. Furthermore, the future electrofishing surveys in salmonid rivers of remote areas could combine two purposes; the main one on salmonids (i), and the added check of gills for glochidia (ii) – an approach that is advisable also in the sense of limited science funds.

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Table 1. Naked-eye classifications (infected or non-infected by *M. margaritifera*) and microscopic counts for number of glochidia, with fish and glochidia lengths, for brown trout individuals electrofished from the Rivers Jukuanoja and Koivuoja between June and August 2011. Incorrect naked-eye classifications are marked with * and the fish with freshly attached small glochidia with a border lined box.

The River Jukuanoja					The River Koivuoja				
Date	Fish length (mm)	Naked-eye classification	Number of glochidia	Mean length ± S.E. of glochidia (µm)	Date	Fish length (mm)	Naked-eye classification	Number of glochidia	Mean length ± S.E. of glochidia (µm)
June 9	77	inf.	176	291 ± 9.1	June 9	134	inf.	1112	289 ± 10.1
	109	inf.	165	250 ± 9.6		151	inf.	67	272 ± 9.9
	147	inf.	241	258 ± 9.1		113	inf.	558	287 ± 10.9
	77	non-inf.	0			160	inf.	1674	286 ± 7.4
	78	inf.	105	247 ± 8.0					
June 27	94	inf.	16	319 ± 9.4	June 28	162	non-inf. *	3	333 ± 36.5
	132	inf.	1460	353 ± 9.6		77	inf.	108	357 ± 10.1
	110	inf.	406	330 ± 9.0		139	non-inf. *	19	363 ± 11.7
	90	inf.	126	330 ± 11.6		138	non-inf. *	1	316
						97	non-inf.	0	
July 20	115	non-inf. *	11	296 ± 8.0	July 21	158	non-inf. *	1	456
	112	inf.	6	392 ± 18.5		169	non-inf. *	1	439
	168	non-inf.	0			86	non-inf.	0	
	54	non-inf.	0			146	non-inf.	0	
	150	inf.	128	395 ± 9.9					
August 2	216	non-inf.	0		August 3	138	non-inf.	0	
	112	non-inf.	0			150	non-inf.	0	
	63	non-inf.	0			157	non-inf.	0	
	59	non-inf.	0			106	non-inf.	0	
August 31	121	non-inf. *	16	74 ± 6.3	August 31	148	non-inf.	0	
	126	non-inf. *	265	81 ± 2.9		143	non-inf.	0	

	71	non-inf. *	235	77 ± 4.7	66	non-inf.	0
	134	non-inf. *	329	81 ± 2.9	145	non-inf.	0
					65	non-inf.	0

Table 2. Naked-eye scores for intensity (from 0 = no glochidia to 5 = very high number of glochidia, - = no score) of *M. margaritifera* infection by independent observers (O1, O2, O3), with mean of the scores, and microscopic counts for number of glochidia, with fish and glochidia lengths, for brown trout individuals electrofished from the Rivers Majovanoja and Pahkaoja between June 3 and 6, 2012 and 2013. Incorrect naked-eye classifications are marked with *. Association between the means of the naked-eye scores and microscopic counts was highly significant (see Results).

	The River Majovanoja							The River Pahkaoja						
	Fish length	Naked-eye scores				Number	Mean length ± S.E.	Fish length	Naked-eye scores				Number	Mean length ± S.E.
Year	(mm)	O1	O2	O3	Mean	of glochidia	of glochidia (µm)	(mm)	O1	O2	O3	Mean	of glochidia	of glochidia (µm)
2012	69	0 *	1	0 *	0.33	4	219 ± 8.8	75	1	1	1	1.00	14	-
	68	3	3	2	2.67	141	202 ± 26.3	122	3	3	2	2.67	399	-
	70	3	3	4	3.33	143	246 ± 17.5	92	1	1	1	1.00	23	-
								57	0	1 *	0	0.33	0	
								52	0	1 *	0	0.33	0	
2013	138	2	1	-	1.50	1	228	74	2	1	-	1.50	23	233 ± 7.9
	121	1 *	0	-	0.50	0								
	72	5	5	-	5.00	387	296 ± 7.1							
Mean		2.33	2.17	2.00	2.20	113			1.17	1.33	0.80	1.12	77	

Table 3. Naked-eye scores for intensity (from 0 = no glochidia to 5 = very high number of glochidia) of *M. margaritifera* infection by two independent observers (O1, O2), with mean of the scores, and microscopic counts for numbers of glochidia, with fish and glochidia lengths, in brown trout exposed to *M. margaritifera* glochidia in laboratory. In addition to these 12 fish, there were 28 other fish inspected, with no glochidia found and having correctly scored as 0 by both observers. The exposure took place in September 19 2014, and fish were scored on July 13 2015. Incorrect naked-eye classifications are marked with *. Association between the means of the naked-eye scores and microscopic counts was highly significant (see Results).

Fish		Naked-eye scores			Number	Mean length ± S.E.
No.	length (mm)	O1	O2	Mean	of glochidia	of glochidia (µm)
1	114	5	5	5.00	1669	405 ± 6.8
2	118	5	4	4.50	1459	368 ± 10.3
3	109	5	4	4.50	1290	-
4	120	4	4	4.00	908	394 ± 6.9
5	126	4	4	4.00	782	396 ± 6.5
6	121	4	4	4.00	735	409 ± 5.4
7	115	5	3	4.00	596	-
8	121	2	2	2.00	184	340 ± 10.8
9	121	1	1	1.00	71	361 ± 9.0
10	128	1	1	1.00	47	339 ± 10.6
11	109	0 *	0 *	0	1	-
12	133	0	1 *	0.5	0	

Table 4. The 40 rivers electrofished in June 3–14 2012–2013 with total fishing effort (f) and numbers of brown trout caught (N_{fish}). The mean naked-eye scores for intensity (from 0 = no glochidia to 5 = very high number of glochidia, - = no score) of *M. margaritifera* infection in the fish are shown by each

observer (O1, O2, O3) and per each river, as well as both number of infected fish ($N_{infected}$) and prevalence and intensity of infection observed in microscopic examination. Rivers with fish confirmed to harbour *M. margaritifera* infection, i.e. the 'new' *M. margaritifera* populations, are marked with *.

					Naked-eye scores				Laboratory examination		
No.	River	Fishing year	f (min)	N_{fish}	O1	O2	O3	Mean	$N_{infected}$	Prevalence (%)	Mean intensity ± S.E.
1	Aimojoki	2012 & 2013	165	9	0	0	-	0	0	0	
2	Elehvänoja	2012	45	5	0	0	-	0	0	0	
3	Hietajoki 1	2013	60	5	0.20	0.60	-	0.40	0	0	
4	Jaaskamonoja	2012 & 2013	195	9	0	0	-	0	0	0	
5	Karhuoja	2012	45	1	0	0	0	0	0	0	
6	Kisosjoki *	2012	135	19	0.05	0.21	0.11	0.12	3	16	3.0 ± 0.6
7	Koiraoja	2012	90	2	0	0	-	0	0	0	
8	Kostonlammenoja *	2013	135	14	0.21	0.07	-	0.14	6	43	12.7 ± 4.1
9	Kutinjoki	2012 & 2013	180	5	0	0	-	0	0	0	
10	Kylmävaaranpuro	2012	30	6	0	0	-	0	0	0	
11	Latvajoki 1	2012	120	5	0.20	0	0	0.07	0	0	
12	Lohioja	2013	60	2	0	0	-	0	0	0	
13	Loukusanjoki	2012 & 2013	120	8	0	0	0.20	0.07	0	0	
14	Lukkarinoja *	2012	60	5	0.80	0.60	0.80	0.73	1	20	183
15	Ohtaoja	2013	90	5	0	0.20	-	0.10	0	0	
16	Oudonjoki	2012	30	4	0	0	-	0	0	0	
17	Paljakkaoja	2012	60	5	0.20	0	0	0.07	0	0	
18	Pirinoja	2012	45	5	0	0	0	0	0	0	
19	Rääpysoja	2012 & 2013	80	4	0	0	-	0	0	0	
20	Siiranjoki	2012 & 2013	195	13	0	0	-	0	0	0	
21	Tutuoja	2012	55	5	0	0	0	0	0	0	
22	Ylä-Haapuanoja	2013	90	5	0	0	-	0	0	0	

23	Ahmaoja	2012	75	0
24	Askanjoki	2012 & 2013	90	0
25	Harjajoki	2013	150	0
26	Hietajoki 2	2013	60	0
27	Kylmäjoki	2012	90	0
28	Kylmäluomanoja	2013	30	0
29	Lahnasenoja	2012	60	0
30	Latvajoki 2	2012	45	0
31	Martinjoki	2013	90	0
32	Mäntyjoki	2012 & 2013	150	0
33	Puhosjoki	2013	120	0
34	Riitainjoki	2012	30	0
35	Tervaoja	2012	60	0
36	Tolpanoja	2013	60	0
37	Visaoja	2012	30	0
38	Välijoki	2013	30	0
39	Vääränoja	2013	30	0
40	Väätäjänoja	2013	30	0

Figure 1. Map of the River Iijoki drainage with its main tributaries. The star symbols J, K, M and P depict the locations of the Rivers Jukuanoja, Koivuoja, Majovanoja and Pahkaoja, respectively, where the electrofishing method was tested in 2011–2012. L is the River Livojoki, where *M. margaritifera* glochidia were collected for laboratory study. The 40 dots are the survey points of the rivers where the method was applied in 2012–2013; the Rivers Kisosjoki, Kostonlammenoja and Lukkarinoja are marked as N1, N2 and N3, respectively. The inset map of northern Europe shows Finland in grey and the River Iijoki catchment in black. For conservation of *M. margaritifera*, the exact coordinates for the study rivers are not given, but they are available from the authors.

Figure 2. Categories 1, 2, 3, 4 and 5, respectively, for the intensity of *M. margaritifera* infection on gills of brown trout shown in microscopic photos. Category 0 indicates that any larva was not observed on the gills.


Atlantic
Ocean
L
J
N2
M
P
K
N1
N3
30 km