JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
314

Jouni Salonen

# The Role of Salmonid Fishes in Conservation of the Endangered Freshwater Pearl Mussel (*Margaritifera margaritifera*)

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa YAA303, huhtikuun 29. päivänä 2016 kello 12.

Academic dissertation to be publicly discussed, by permission of the Faculty of Mathematics and Science of the University of Jyväskylä, in building Ambiotica, hall YAA303, on April 29, 2016 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2016

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Publishing Unit, University Library of Jyväskylä



Cover photo: Panu Oulasvirta

URN:ISBN:978-951-39-6602-7
ISBN 978-951-39-6602-7 (PDF)

ISBN 978-951-39-6601-0 (nid.)
ISSN 1456-9701



Jyväskylä University Printing House, Jyväskylä 2016

## ABSTRACT

Salonen, Jouni
The role of salmonid fishes in conservation of the endangered freshwater pearl mussel (*Margaritifera margaritifera*)
Jyväskylä: University of Jyväskylä, 2016, 48 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 314)
ISBN 978-951-39-6601-0 (nid.)
ISBN 978-951-39-6602-7 (PDF)
Yhteenveto: Lohikalojen rooli jokihelmisimpukan (*Margaritifera margaritifera*) suojelussa
Diss.

The abundance of freshwater pearl mussels (*Margaritifera margaritifera*) has declined widely during the past century, and new conservation initiatives are needed. This thesis focused on the relationship between *M. margaritifera* and its salmonid host required for reproduction of this species. First, by exposing fish experimentally to glochidium larvae of *M. margaritifera*, different *M. margaritifera* populations were shown to demonstrate strong differences in their ability to parasitize different salmonid species. Atlantic salmon was clearly a better host for mussels in large river channels, whereas in small headwater tributaries brown trout was the best, or the only suitable, host. These findings provide a previously unrecognised explanation for the collapse and the lack of recruitment especially of the salmon-specific *M. margaritifera* populations; a high proportion of large salmon rivers were dammed for hydropower production in the 1960s, which prevented the migration of salmon and thus left *M. margaritifera* without the appropriate host in these rivers. Furthermore, an invasive salmonid, brook trout, was widely introduced to small tributaries above the dams in the past, but in this study was shown to be an unsuitable host for *M. margaritifera*. Thus, and due to the tendency of brook trout to replace native brown trout, the spread of brook trout is an additional threat to *M. margaritifera*. An indication of local adaptation of *M. margaritifera*, i.e. higher infectivity in sympatric salmonid host strain than in allopatric populations of the same species, was also detected. Finally, a new, non-destructive approach to search for *M. margaritifera* populations, involving electrofishing and quick visual examination of the gills of captured salmonids, revealed the occurrence of 3 previously unknown populations. The results of this thesis highlight the importance of taking into account the roles of salmonid fish in future efforts to search, protect and restore freshwater pearl mussel populations.

Keywords: Freshwater pearl mussel; glochidium parasitism; host fish; invasive species; *Salmo salar*; *Salmo trutta*; *Salvelinus fontinalis*.

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## LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I–IV.

I planned articles I–II together with Jouni Taskinen, while the ideas for III–IV were mainly mine. Most of the field experiments in the articles were prepared by fisheries planners Pirkko-Liisa Luhta and Eero Moilanen (Natural Heritage Services Finland) and the laboratory experiments by Jouni Taskinen, while I took the main responsibility for the final field and laboratory work in every experiment. The statistical analyses were conducted by me, with the help of Timo J. Marjomäki in I and III–IV and Jouni Taskinen in III. I wrote the bases of manuscripts I and III, while the first draft for II came from Jouni Taskinen and IV was equally written by both authors. During the revision stages, the articles were finalized jointly with the co-authors.

I Salonen J.K., Luhta P.-L., Moilanen E., Oulasvirta P., Turunen J. & Taskinen J. 2016. Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) differ in their suitability as a host for the endangered freshwater pearl mussel (*Margaritifera margaritifera*) in northern Fennoscandian rivers. Submitted manuscript.

II Taskinen J. & Salonen J.K. 2016. Origin matters: freshwater pearl mussels show adaptation to sympatric salmonid host strains. Manuscript.

III Salonen J.K., Marjomäki T.J. & Taskinen J. 2016. An alien fish threatens an endangered parasitic bivalve: the relationship between brook trout (*Salvelinus fontinalis*) and freshwater pearl mussel (*Margaritifera margaritifera*) in northern Europe. *Aquatic Conservation: Marine and Freshwater Ecosystems*, doi: 10.1002/aqc.2614.

IV Salonen J.K. & Taskinen J. 2016. Electrofishing as a new method to search for unknown populations of the endangered freshwater pearl mussel *Margaritifera margaritifera*. *Aquatic Conservation: Marine and Freshwater Ecosystems*. In press.

# 1 INTRODUCTION

## 1.1 Habitat preferences and life cycle of the freshwater pearl mussel

The freshwater pearl mussel (*Margaritifera margaritifera*, hereafter FPM), an endangered bivalve belonging to the order of Unionoida, occurs on both sides of the Atlantic Ocean in the Northern hemisphere (Athearn and Clarke 1962, Bauer 1986, Ziuganov *et al.* 1994, Young *et al.* 2001, Geist and Auerswald 2007, Geist 2010, Lopes-Lima *et al.* 2016). It can colonize fluvial habitats from small streams to large rivers, but is generally more frequent in cold and pristine 0.3–0.4 m deep waters which are low in vegetation and nutrients, poor in calcium and rich in oxygen, the bottom of which consists of patches of rocks, coarse sand and fine gravel and which have current speed of 0.3–0.5 $ms^{-1}$ (Clarke and Berg 1959, Young and Williams 1983a, Valovirta 1993, Beasley and Roberts 1999, Álvarez-Claudio *et al.* 2000, Hastie *et al.* 2000a, Geist and Auerswald 2007, Dolmen and Kleiven 2008, Outeiro *et al.* 2008, Ostrovsky and Popov 2011, Degerman *et al.* 2013, Jung *et al.* 2013, Varandas *et al.* 2013, Lopes-Lima *et al.* 2016). Reflecting these habitat requirements, this mollusc is often considered an indicator of good water quality and a healthy, unpolluted ecosystem which is at least close to natural state (e.g. Ziuganov *et al.* 1994, Geist 2010).

As a typical characteristic in the life cycle of a unionoid mussel (see e.g. Bauer 1994, Wächtler *et al.* 2001, Barnhart *et al.* 2008, Lopes-Lima *et al.* 2016), the reproduction process of FPM (Fig. 1) is multiphase and includes glochidial parasitism in a host fish (Young and Williams 1984a, Bauer 1988, Hruška 1992, Ziuganov *et al.* 1994, Hastie and Young 2001, 2003, Ieshko *et al.* 2009, Geist 2010). The process begins in late summer, when male mussels release their sperm into the river. The sperm is then inhaled by female mussels to fertilize the eggs in their marsupial gills. After several weeks, when the fertilized eggs have developed to round glochidium larvae of 50–70 µm diameter, the glochidia are shed into the water by the females (Young and Williams 1984a, Bauer 1987b, Pekkarinen and Valovirta 1996, Schmidt and Vandré 2010, Scheder *et al.* 2011, Denic *et al.* 2015), after which the glochidia have to reach and attach to appropriate host fish for further development. Unlike the glochidia of many other mussel species, which may successfully parasitize several fish species and attach either on gills, fins or skin of the host (see e.g. Bauer 2001, Jansen *et al.* 2001, Wächtler *et al.* 2001, Barnhart *et al.* 2008), successful encystment of FPM glochidia has been confirmed only on the gills of Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in Europe (e.g. Young and Williams 1984a, 1984b, Bauer 1987c, Hastie and Young 2001), whereas in North America brook trout (*Salvelinus fontinalis*) has also been suggested to be a suitable FPM host (Clarke and Berg 1959, Athearn and Clarke 1962, Smith 1976). In addition to the salmonid host specificity, the long duration of the parasitic stage and the remarkable growth of glochidia during that stage distinguish FPM from many other mussel species; in the northernmost range of FPM, the glochidia remain attached on the host fish for almost a year, from early autumn to late summer of the following year (Young and Williams 1984b, Hruška 1992, Hastie and Young 2001, Ieshko *et al.* 2009, Schmidt and Vandré 2010), while in southern latitudes the parasitic life stage may end several months earlier (Cunjak and McGladdery 1991, Ziuganov *et al.* 1994, Eybe *et al.* 2015). Nevertheless, during that stage the glochidia metamorphose into slightly ovoid juvenile mussels of 400–500 µm length (Young and Williams 1984b, Bauer 1987b, 1987c, Bauer and Vogel 1987, Schmidt and Vandré 2010, Denic *et al.* 2015), after which the juveniles leave the host and penetrate into the river bottom substratum for several years (Young and Williams 1983a, Bauer 1988, San Miguel *et al.* 2004, Geist and Auerswald 2007, Bolland *et al.* 2010, Ostrovsky and Popov 2011), presumably to hide from predators (see Zimmerman *et al.* 2003). The mortality of FPM is very high during these early life stages even in optimal conditions; it is estimated that 95–99 % of glochidia shed into the water do not reach a suitable host and die, 90–95 % of the glochidia attached to a suitable host die before completing metamorphosis, and a further 95 % of young mussels are lost between detachment from the host and establishment in the bottom substratum (Young and Williams 1984a, Hastie and Young 2001, Preston *et al.* 2007, Schmidt and Vandré 2010). However, after these critical bottleneck stages, the benthic life of an adult FPM may last as long as 2 centuries in Fennoscandia (Ziuganov *et al.* 2000, Helama and Valovirta 2008, Österling *et al.* 2010, Dunca *et al.* 2011), while elsewhere the maximum lifespan of FPM seems to be less than 100 years (Bauer 1983, Young and Williams 1984a, Bauer 1987a, 1992, Hruška 1992, Ziuganov *et al.* 2000, San Miguel *et al.* 2004, Varandas *et al.* 2013).

In addition to the long lifespan in the reproductive stage after maturation at the age of 12–20 years (Young and Williams 1983b, 1984a, Bauer 1987a), the considerable mortality of glochidia and juveniles in the early life stages is also compensated by enormous fecundity as an adult mussel produces millions of glochidia annually (Young and Williams 1984a, Bauer 1987a). Furthermore, FPM is able to change sex or become hermaphroditic to ensure reproduction at low mussel densities (Bauer 1987a, Grande *et al.* 2001). FPM may also reproduce twice a year in extraordinarily warm summers (Scheder *et al.* 2011), but e.g.

anthropogenic disturbances may lead populations or individuals to skip the annual reproduction (Bauer 1998, Scheder and Gumpinger 2008).

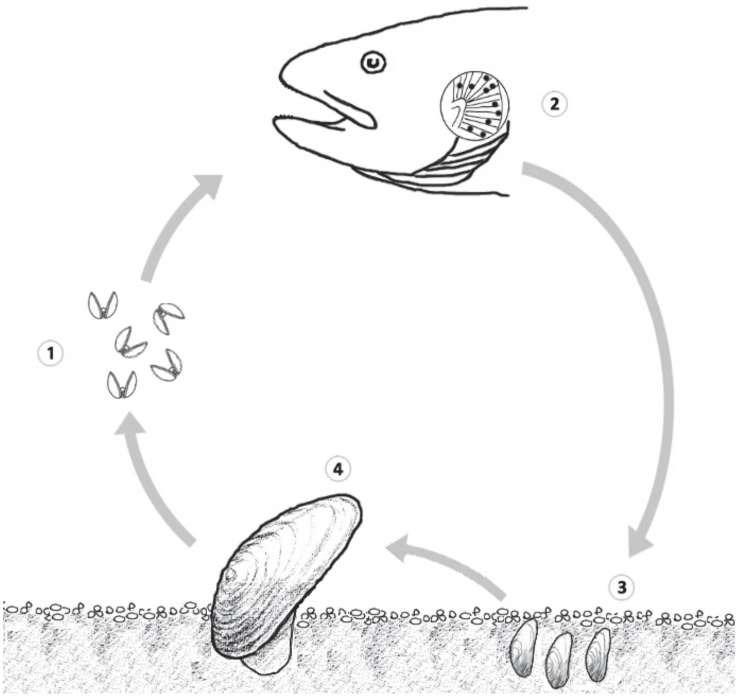


FIGURE 1 Life cycle of the freshwater pearl mussel. Tiny glochidium larvae (1) are released into the water, after which they attach to the gills of salmonid fish for several months (2). In the host the larvae metamorphose into juvenile mussels (3), which spend their first years within the river substratum and become mature, adult mussels (4) at the age of 12–20 years. Figure modified from Valtonen *et al.* (2012) p. 220 with kind permission of Gaudeamus.

## 1.2 The collapse of the freshwater pearl mussel

FPM used to be abundant in European salmon and brown trout rivers in the past, but has strongly declined in the 20th century, being now extinct or close to extinction with only a small number of viable populations or subpopulations remaining in the formerly dense mussel beds (e.g. Young and Williams 1983b, Bauer 1986, Ziuganov *et al.* 1994, Beasley *et al.* 1998, Cosgrove *et al.* 2000, Young *et al.* 2001, Dolmen and Kleiven 2008, Scheder and Gumpinger 2008, Oulasvirta 2011, Varandas *et al.* 2013, Simon *et al.* 2015, Lopes-Lima *et al.* 2016). The decline has also been dramatic in Finland; it is estimated that at the beginning of the 20th century there were over 200 viable FPM populations, while today a FPM population with recruitment can be found only in a few dozen rivers (Valovirta

2006, Geist 2010, Oulasvirta 2010, 2011). The European FPM is now classified as a critically endangered species (Cuttelod *et al.* 2011).

The collapse of FPM is generally suggested to be the result of anthropogenic environmental perturbations such as eutrophication, contamination and siltation, which have caused the loss of suitable habitats (Bauer 1983, 1986, 1988, Valovirta 1993, Ziuganov *et al.* 1994, Beasley *et al.* 1998, Cosgrove *et al.* 2000, Young *et al.* 2001, Dolmen and Kleiven 2008, Scheder and Gumpinger 2008, Geist 2010, Österling *et al.* 2010, Oulasvirta 2010, Varandas *et al.* 2013, Gosselin 2015, Simon *et al.* 2015, Lopes-Lima *et al.* 2016). In addition, river dredging and channelization, as well as loss of host fish via e.g. pollution, overfishing and hydropower dam construction, have had evident impacts on the collapse of FPM (Ziuganov *et al.* 1994, Beasley *et al.* 1998, Cosgrove *et al.* 2000, Cosgrove and Hastie 2001, Hastie and Cosgrove 2001, Makhrov *et al.* 2011, Young *et al.* 2001, Reis 2003, Dolmen and Kleiven 2008, Geist 2010, Oulasvirta 2010, 2011, Österling and Högberg 2014, Simon *et al.* 2015, Lopes-Lima *et al.* 2016). Harvesting of pearls has also depleted FPM populations (Young and Williams 1983b, Bauer 1986, Ziuganov *et al.* 1994, Beasley *et al.* 1998, Beasley and Roberts 1999, Makhrov *et al.* 2011, Cosgrove *et al.* 2000, 2014), despite the fact that it has been widely banned, for example since 1955 in Finland (Oulasvirta 2011). Natural enemies of FPM are unlikely to have caused the decline, as FPM has hardly any (Bauer 1987a, Ziuganov *et al.* 1994); only muskrat, crayfish and eel have occasionally been found to consume mussels (Zahner-Meike and Hanson 2001, Geist 2010).

Many conservation strategies for the restoration of FPM populations have been developed; habitat protection and restoration (Ziuganov *et al.* 1994, Cosgrove and Hastie 2001, Bolland *et al.* 2010, Geist 2010, Horton *et al.* 2015) probably are the primary strategies but methods such as reintroduction of mussels (Young and Williams 1983b, Valovirta 1993, Beasley and Roberts 1999, Bolland *et al.* 2010, Geist 2010), release of glochidia-infected fish (Buddensiek 1995, Geist *et al.* 2006, Thomas *et al.* 2010, Simon *et al.* 2015) and artificial culture of mussels (Buddensiek 1995, Preston *et al.* 2007, Geist 2010, Schmidt and Vandré 2010, Thomas *et al.* 2010, Gum *et al.* 2011, Simon *et al.* 2015) are also widely used. However, despite the conservation activities, increased freshwater mussel research (see e.g. Thomas *et al.* 2010) and the conservation status of FPM afforded by EU directives and national legislation (see Lopes-Lima *et al.* 2016), the decline of FPM populations has continued and even steepened. Furthermore, there are predictions of more populations becoming extinct in the future (Bauer 1983, Valovirta 1990, Beasley *et al.* 1998). For example, illegal pearl fishing is still taking place in Scotland (Cosgrove *et al.* 2014) and probably elsewhere, but most alarmingly, absence or low number of juvenile mussels has been detected in many of the remaining populations (Valovirta 1990, 1993, Cosgrove *et al.* 2000, Hastie *et al.* 2000b, Reis 2003, Österling *et al.* 2008, Outeiro *et al.* 2008, Oulasvirta 2011, Varandas *et al.* 2013, Simon *et al.* 2015), indicating that the reproduction of populations has stopped or does not lead to successful recruitment. Thus, these populations will not be able to persist in the long run. The above-mentioned anthropogenic factors are suggested as the main reasons for the recruitment failures, as adult mussels are more tolerant than juveniles of deteriorated conditions (Hastie *et al.* 2000a, Helama and Valovirta 2007). In addition, Geist and Auerswald (2007) showed that poor stream bed quality limits the recruitment of FPM, and other studies have also suggested that the glochidium (Taskinen *et al.* 2011) and juvenile stages (Buddensiek 1995, Österling *et al.* 2008, 2010, Schmidt and Vandré 2010, Dunca *et al.* 2011) are the critical periods when a high proportion of FPM individuals are lost if the conditions are not optimum. However, as the number of successful conservation strategies in which these facts have been taken into account has remained limited (Simon *et al.* 2015, Lopes-Lima *et al.* 2016), there may also be other, as yet unidentified factors behind the recruitment failures.

## 1.3 The interactions between the freshwater pearl mussel and host fish

The interactions between salmonids and FPM are under debate. Negative effects of FPM parasitism for the host, such as mortality (Meyers and Millemann 1977, Taeubert and Geist 2013), respiratory cost (Thomas *et al.* 2014) and reduced ability to move (Taeubert and Geist 2013) or feed (Österling *et al.* 2014) have been reported. On the other hand, the functional role of mussels in river ecosystems is undoubted; they filter plankton, bacteria, algae, detritus and even dissolved organic matter from the water column for feeding, thus purifying the water but also biodepositing these materials as pseudofaeces to the river bottom, thereby producing a nutrient-rich and easily assimilated food source for benthic invertebrates and thus supporting also salmonid production (Ziuganov *et al.* 1994, Pusch *et al.* 2001, Vaughn and Hakenkamp 2001, Howard and Cuffey 2006, Christian *et al.* 2008, Vaughn *et al.* 2008). Moreover, according to Vaughn *et al.* (2008), mussels provide physical structure via stabilization and bioturbation of sediments, and these effects support, link and influence multiple trophic levels of fluvial ecosystems. Therefore, the relationship between FPM and their salmonid hosts has occasionally been suggested to be symbiotic rather than parasitic (Nezlin *et al.* 1994, Ziuganov 1994, 2005). Furthermore, Ziuganov (2005) has shown that even the FPM infection may be beneficial for salmonid individuals by strengthening them and helping them to survive from asphyxia and hook wounds with higher probability than the uninfected conspecifics.

As an FPM host, the suitability of salmon and brown trout, in addition to different strains of these species, has generally been considered equal (Bauer 1987c). However, this impression has been questioned recently, because differences in the suitability of either of these well-known hosts for certain FPM populations have been found in Norway (Karlsson *et al.* 2014) and Sweden (Österling and Wengström 2015). Furthermore, Taeubert *et al.* (2010) and Österling and Larsen (2013) demonstrated differences between different strains of brown trout in their suitability as FPM host. Thus, for conservation of FPM, and for better understanding the interactions between FPM and salmonids, there is a need for wider investigation of the potential host specificity, especially in northernmost Europe which harbours the most important FPM populations in terms of high genetic diversity (Geist and Kuehn 2008, Geist *et al.* 2010).

Furthermore in northern Europe and especially in Finland, the rise of hydropower production during the 20th century (e.g. Karppinen *et al.* 2002, Erkinaro *et al.* 2011, Marttila *et al.* 2014) led to intensive construction of dams on large river systems so that today there are only 2 undammed large river channels (Simojoki and Tornionjoki) within the Finnish part of the Baltic Sea drainage. Because equipping the hydropower plants with fishways has been very rare (Karppinen *et al.* 2002, Erkinaro *et al.* 2011, Marttila *et al.* 2014), the hydro-electricity production has caused the occurrence of anadromous salmonids to collapse as the dams have prevented their natural spawning migration to these rivers. The lack of salmonids in dammed rivers has been compensated by annual stocking of farmed brown trout above the dams, the fish originating from both local (sympatric with FPM) and non-local (allopatric with FPM) strains (Luhta and Moilanen 2006, Hiltunen 2010). Invasive brook trout and rainbow trout have also been introduced e.g. in the River Iijoki area to support the traditional activity, salmonid fishing (Luhta and Moilanen 2006, Hiltunen 2010). However, Atlantic salmon have been stocked only to estuaries near the sea, and thus below the dams (Erkinaro *et al.* 2011, Marttila *et al.* 2014), where they cannot migrate to the rivers. Above the dams, attempts have been made to maintain salmon populations via sporadic egg stocking and translocating adult salmon to upstream (see e.g. Erkinaro *et al.* 2011). However, the salmonid species in the former spawning grounds of Atlantic salmon has in practice changed from salmon to brown trout due to the dam construction and the subsequent stockings. This change raises a question of whether the FPM populations that live in the former Atlantic salmon habitats in the dammed rivers are able to use the substitute salmonid, brown trout, as their host.

While the above-mentioned anthropogenic replacement of salmon by brown trout has mainly occurred in large channels since salmon generally prefers the large river sections at the lower reaches of rivers (Baglinière *et al.* 1994, Erkinaro 1995, Klemetsen *et al.* 2003, Johansen *et al.* 2005), the original salmonid communities in smaller headwater streams may also have become disturbed in the aftermath of hydropower dam construction. First, the spread of non-local brown trout may have decreased the proportion of the brown trout from the local, resident strain in these fish communities, and thus challenged the mussels' ability to parasitize the stocked non-local brown trout. Secondly, the stocking of invasive brook trout has led to an unexpected and undesired replacement of the native European brown trout by this invader, especially in small tributary streams (Korsu *et al.* 2007, Spens *et al.* 2007, Öhlund *et al.* 2008). Thus, if the non-local brown trout or the invasive brook trout are not appropriate hosts for FPM, unlike the original fish, the invasion of these fish may cause also the FPM populations in the headwater streams to lose their necessary host fish. Previous studies on both these subjects are scarce and contradictory. A higher infectivity of glochidia in brown trout originating from a FPM habitat than elsewhere was found by Taeubert *et al.* (2010) and Jung *et al.* (2013), while in the study of Österling and Larsen (2013) a non-local brown trout strain was the most intensively infected fish. Brook trout is suggested to be a suitable FPM host in the USA (Clarke and Berg 1959, Athearn and Clarke 1962, Smith 1976), but not in Germany (Bauer 1987c) or Austria (Jung *et al.* 2013). In northern Fennoscandia, where stocking of brook trout has been most intensive, neither the host suitability nor the distribution of this invader in FPM rivers has been investigated previously.

Generally in parasitology, adaptation of parasites to sympatric, local host population is often found (Ebert 1994, Kaltz and Shykoff 1998, Saarinen and Taskinen 2005, Greischar and Koskella 2007), but so-called maladaptation, i.e. better infectivity of an allopatric host population has also been occasionally detected (Kaltz and Shykoff 1998, Kaltz *et al.* 1999, Oppliger *et al.* 1999). Furthermore, invasive species are usually less parasitized by the parasites of their target area in comparison with the native hosts of the area (Torchin *et al.* 2003, Vilà *et al.* 2005), and this freedom from parasites is often suggested to further support the invasions (enemy release hypothesis; see e.g. Sax and Brown 2000, Mitchell and Power 2003). However, there are also examples of invader being as good a host as the native species for the native parasites (Poulin and Mouillot 2003, Brandner *et al.* 2013). The potential natural (Langefors *et al.* 2001) and acquired (Young and Williams 1984a, Bauer 1987b, Hastie and Young 2001, Dodd *et al.* 2005, Treasurer *et al.* 2006) immune responses of hosts against parasites may also affect host–parasite co-evolution. Moreover, FPM has a much longer lifespan and higher maturation age and hence a longer generation time than the salmonids host, being thus an extraordinary parasite (Kaltz and Shykoff 1998, Gandon and Michalakis 2002, Greischar and Koskella 2007, Geist and Kuehn 2008). The generalizations regarding host–parasite interactions may then not apply to the case of FPM and their hosts, and more research especially into this FPM-host interaction is required to estimate the effects of the spread of non-indigenous salmonid species and strains to FPM habitats.

## 1.4 Mapping the occurrence of the freshwater pearl mussel

Finding possible unknown populations of a vulnerable species and estimating their abundance is important for assessing the current conservation status of the species. Knowledge of current habitats and the distributional range of a species also provides valuable information for activities related to conservation, restoration and management. These conservation challenges apply also to the FPM; despite the worldwide perspective from different types of FPM research conducted since the $16^{th}$ century (Bogan and Roe 2008), and although the attraction of humans to valuable pearls begun even before the Common Era

(Young and Williams 1983b), it is often stated that the occurrence of this endangered mussel is still not mapped thoroughly (e.g. Larsen 2010, Makhrov *et al.* 2011, Ostrovsky and Popov 2011, Oulasvirta 2011). The fact that previously unknown FPM populations have been found quite recently (Álvarez-Claudio *et al.* 2000, Reis 2003, Valovirta 2006, Ostrovsky and Popov 2011, Oulasvirta 2011) supports that statement. One apparent explanation for the incomplete knowledge is the occurrence of FPM in oligotrophic waters that are close to their natural state, meaning that many of the remaining populations are located in remote areas far from human civilization (see e.g. Ostrovsky and Popov 2011, Oulasvirta 2011, Varandas *et al.* 2013, Ieshko *et al.* 2016). Therefore, unexplored rivers and streams in such areas may harbour more unknown FPM populations vulnerable to possible environmental operations by man.

As the mobility of FPM is very low (Young and Williams 1983a), the occurrence of FPM has traditionally been investigated by visual searching. In smaller streams an aquascope (a glass-bottomed box) is usually used (Cosgrove *et al.* 2000, Reis 2003, Valovirta 2006, Österling *et al.* 2008, Ostrovsky and Popov 2011, Oulasvirta 2011, Varandas *et al.* 2013, Simon *et al.* 2015) and in rivers with moderate depth snorkelling is the common method (Oulasvirta 2011, Varandas *et al.* 2013), while in large channels scuba diving may be the only possibility to detect mussels (Oulasvirta 2011). However, certain river characteristics, such as dark or deep water, strong current or stony bottom, may limit the use of these methods (Young and Williams 1983a, Álvarez-Claudio *et al.* 2000, Cosgrove *et al.* 2000, Oulasvirta 2011). Thus any new means to search for FPM would be welcome. One new approach could be to first focus on catching the potential hosts of FPM; presence of glochidia on the gills of captured salmonids could then provide proof of the occurrence of FPM in the river from which the salmonids were caught. Furthermore, if captured and examined non-destructively, fish with FPM glochidia could be released in the river after examination. In fact, this kind of method, involving electrofishing and photographing gills of caught salmonids in order to count the number of encysted glochidia there, was recently developed by Österling (2011). However, the increased size of FPM glochidia in late part of the parasitic stage raises the question whether the glochidia in the gills are detectable even with the naked eye, i.e. without the procedure of anaesthetising or photographing. Thus, the fish could be examined immediately after the catch when they are still stunned due to the electric shock. This examination method could be easily added into any salmonid survey conducted by electrofishing in potential but inadequately investigated FPM areas, and might thus serve in finding unknown FPM populations even outside the area of ordinary FPM research.

## 1.5 Aims of the study

The general objective of this study was to determine whether FPM show population-specific differences in their ability to use certain salmonid species and their different strains as the indispensable host. Then, by connecting the findings to the large scale changes, such as hydropower dam construction, which have affected the occurrence of salmonids in northern rivers, potentially to identify new lines for the conservation of this endangered bivalve.

The first aim of the thesis was to investigate the potential FPM population specific differences in their ability to use either salmon or brown trout as the host (I) by exposing these fishes to FPM glochidia both naturally in FPM rivers and artificially in the laboratory, and comparing the FPM infectivity in them.

The second aim was to investigate, whether FPM populations are specifically adapted to use the local strain of the appropriate salmonid species as the most suitable host, or whether there is possible maladaptation in this host–parasite interaction (II).

The third aim was to study the suitability of the invasive salmonid, brook trout, as a host for FPM (III) in comparison with the confirmed hosts, salmon and brown trout.

Finally, the fourth aim was to develop and test a new, non-destructive and potentially cost-effective method to search for unknown FPM populations by utilizing the host salmonids in the searching (IV).

# 2 MATERIALS AND METHODS

## 2.1 Study areas

All of the articles I–IV included field work in rivers and streams of the River Iijoki catchment (14200 $km^2$, Baltic Sea drainage) in northern Finland (Fig. 2). For I and II, field experiments were also conducted in the main channel of the River Simojoki catchment (3160 $km^2$, Baltic Sea drainage) and in the River Luttojoki and its 2 tributaries belonging to the River Tuloma catchment (21500 $km^2$, Barents Sea drainage) (Fig. 2). The laboratory experiments (I, II and IV) were performed at Konnevesi Research Station (University of Jyväskylä) in central Finland. The authorizations to execute the experiments were acquired from the regional Centres for Economic Development, Transport and the Environment and from the Animal Experiment Board of Finland.

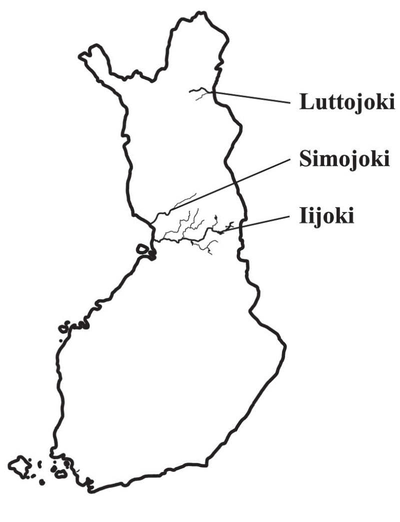


FIGURE 2 The study catchments in northern Finland. The River Luttojoki is a large tributary within the River Tuloma catchment, located mostly in Russia.

## 2.2 Cage experiments

The articles I–III included field experiments in which the suitability of brown trout, Atlantic salmon and brook trout, including different strains of brown trout and salmon (Table 1), as host for certain FPM populations was tested by caging fish in FPM rivers. These experiments were carried out in 4 rivers in 2011, in 5 rivers in 2012 and in 2 rivers in 2013 (Table 1), including both large salmon rivers and small headwater streams colonized by brown trout as the only salmonid. The cylindrical, steel cages containing fish were placed close to the known FPM habitats, and 2–4 replicate cages per fish group were used. Each experiment was started in early autumn, shortly before the shedding of glochidium larvae by female mussels, to expose the fish to naturally shed glochidia. To allow enough time for exposure and to stabilize the number of FPM glochidia (there is a general decrease in the number of glochidia during the first few days after infection; see e.g. Young and Williams 1984b, Bauer and Vogel 1987, Jansen *et al.* 2001, Jung *et al.* 2013, Österling and Larsen 2013) and to allow the successfully attached glochidia to grow, the cages were not removed earlier than 1–2 months after the start of the caging.

The fish used in the experiments were farmed, except for certain strains (Table 1) which are apparently not farmed anywhere. Wild fish of these strains were caught by electrofishing several weeks before the caging, and well before the season of FPM shedding glochidia, to exclude the potential effect of earlier FPM exposure on the results. Furthermore, use of juvenile 0+ fish was favoured to ensure the fish were not exposed to FPM in earlier years and thus not developed immunity against FPM glochidia (see e.g. Young and Williams 1984a, Bauer 1987b, Hastie and Young 2001, Treasurer *et al.* 2006).

The execution of the experiments went as expected, except for an unavoidable incident when one cage was found damaged, probably by some animal, and all fish had escaped. Otherwise only minor mortality and disappearance of fish was detected. Fish were not fed during the caging.

TABLE 1 The salmonid species and strains used in the cage experiments in different FPM rivers. The Rivers Hanhioja and Kolmosjoki belong to the River Tuloma catchment and Simojoki to the River Simojoki catchment, while the other rivers are located in the River Iijoki catchment. The Iijoki salmon and brown trout strains have been maintained in hatcheries during the decades after hydropower dam construction in the Iijoki area. *w* = wild fish.

| | 2011 | | | | 2012 | | | | | 2013 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fish species and strain | Koivuoja | Lohijoki | Portinjoki | Livojoki | Ala-Haapuanoja | Porraslammenoja | Livojoki | Hanhioja | Kolmosjoki | Livojoki | Simojoki |
| Brown trout Iijoki | • | • | • | • | • | • | • | | | • | • |
| Brown trout Rautalampi | | | | • | • | • | • | | | | |
| Brown trout Kitkajoki | | | | • | | | • | | | | |
| Brown trout Lohijoki, *w* | | • | • | | | | | | | | |
| Brown trout Portinjoki, *w* | | • | • | | | | | | | | |
| Brown trout Ala-Haapuanoja, *w* | | | | | • | | | | | | |
| Brown trout Porraslammenoja, *w* | | | | | | • | | | | | |
| Brown trout Hanhioja, *w* | | | | | | | | • | • | | |
| Brown trout Kolmosjoki, *w* | | | | | | | | • | • | | |
| Brown trout Luttojoki, *w* | | | | | | | | • | • | | |
| Atlantic salmon Iijoki | • | • | • | • | • | • | • | | | • | • |
| Atlantic salmon Simojoki, *w* | | | | | | | | | | • | • |
| Atlantic salmon Tornionjoki | | | | | | | | | | • | • |
| Brook trout | | | | | | • | | | | | |

## 2.3 Laboratory experiments

In the laboratory experiments (I, III and IV), fish were artificially exposed to FPM glochidia. For these exposures, conducted annually during 2011–2014 with FPM of different origin (Table 2), the glochidia were collected following the method used earlier also by Young and Williams (1984a), Bauer (1987a) and Scheder and Gumpinger (2008). First, several mussels were picked from the stream and placed in plastic 5–10 l buckets filled with river water. Probably due to the stress of reduced oxygen concentration (Young and Williams 1984a, Bauer 1987a) or as a response to increased water temperature (Hastie and Young 2003), the female mussels shed their glochidia within 30 minutes. After the shedding, the mussels were returned to the river, and the glochidial suspension with additional water and oxygenators was quickly transported to Konnevesi Research Station. This procedure was carried out in early autumn, shortly before the natural shedding time, to obtain fully developed glochidia.

In the laboratory, the fish (Table 2), obtained from fish farms and usually allocated into 2–4 replicate tanks, were exposed to the collected glochidia. For the exposure, the water flow in the tank system was turned off, after which the volume of each 163 l fish tank was decreased to 70 l. Then, 2 l (2011), 1 l (2012), 0.5 l (2013) or 1.5 l (2014) of the FPM suspension was added to each tank. The water flow was kept closed during the 1 h exposure, and the water in the tanks was gently stirred to keep both the fish and the glochidia moving and thus enable even exposure of the fish. Successful infection was confirmed a day after the procedure by examining several fish for FPM glochidia.

Fish were fed with a commercial fish feed during the experiments. Overall, the experiments were carried out successfully, although a couple of unexpected incidents took place. In 2012, predation by brook trout on other salmonids was detected, and thus the brook trout had to be moved to separate tanks. In 2013, a fish group was lost probably due to jumping out from the inadequately covered tanks.

TABLE 2 The salmonid species and strains and origins of FPM in the laboratory experiments. Due to exceptionally and equally large size of brook trout and Rautalampi, Isojoki and Luutajoki brown trout in the first laboratory experiments, these fish were used only in III. The Rivers Koivuoja, Jukuanoja and Livojoki belong to the River Iijoki catchment, while Luttojoki is located in the River Tuloma catchment. All fish individuals used in the experiments were farmed.

| Fish species and strain | Origin of FPM and the year of exposure | | | |
|---|---|---|---|---|
| | Koivuoja 2011 | Jukuanoja 2012 | Luttojoki 2013 | Livojoki 2014 |
| Brown trout Iijoki | ● | ● | ● | ● |
| Brown trout Rautalampi | ● | | | |
| Brown trout Isojoki | ● | | | |
| Brown trout Luutajoki | ● | | | |
| Atlantic salmon Iijoki | ● | ● | ● | |
| Brook trout | ● | ● | | |

## 2.4 Field surveys

An extensive field survey including electrofishing in more than 40 potential FPM rivers and streams was also carried out. The survey investigated natural FPM infection in wild salmonids (III), but primarily it was conducted to test a new method to search for undiscovered FPM populations by electrofishing salmonids and inspecting them with the naked eye for FPM glochidia (IV). The rivers were electrofished during 2011–2013, using GeOmega FA4 or Paulsen FA4 electrofishing gear usually at 1400 V. The fishing was performed standing on the banks of the rivers when possible, to avoid damaging the possible FPM beds. Two persons, one operating the device and the other one catching the fish with a net, performed the fishing. The capture of fish was followed by a quick naked-eye examination of fish gills by 1–3 observers in the field: in 2011 each observer gently opened the fish operculum and classified the fish as being infected or uninfected by FPM, while in 2012–2013 the intensity of FPM infection in fish was classified on a scale of 0, 1, 2, 3, 4 or 5, where 0 indicated that no glochidia was found, and 5 indicated a very high number of larvae. The fish was then killed and stored on ice to be transported to the laboratory for accurate microscopic examination.

The total time used for fishing varied from 15 minutes to 195 minutes per site, and the preliminary target was to catch 5 juvenile salmonids per occasion. However, there were many rivers where no salmonids were found, while in some rivers salmonids appeared to be numerous and some extra individuals were caught.

## 2.5 Examination of fish

Microscopic examination of fish was performed in similar fashion in the cage (I–III) and laboratory (I, III–IV) experiments, and during the field survey (III–IV). First, if the fish was transported alive to the laboratory, it was killed with a sharp blow on the head, which was followed by measurement of total length and fresh mass. The gills were then dissected using scissors and were placed separately on a large glass plate. Then another large glass plate was taken, the gills were pressed between these two plates, and the number of FPM glochidia on each gill was counted microscopically using about 20 x magnification. Finally, the length of 10 FPM glochidia selected at random was measured per fish, except in 2011 when only the glochidia from brook trout were measured. In the laboratory experiment in 2014, the fish were examined also with the naked eye (the procedure described above) before the microscopic inspection.

## 2.6 Data analysis

In the comparisons between different species or strains as the FPM host (I–III), the most suitable host was considered to be the fish in which the prevalence, abundance and/or intensity of FPM infection (in relation to fish size; I–II), and/or length of encysted FPM glochidia, was greatest. The definitions of these variables and the methods used in statistical analyses are presented in Table 3. Furthermore, the association between occurrence of brook trout and occurrence of FPM (III) was analyzed using $\chi^2$ test. Spearman rank correlation analysis was used for evaluating the potential changes in number and length of attached glochidia in certain species or strains between different examination time points (I, III), and for analyzing the association between naked-eye scores and real number of glochidia (IV). Fisher's combined probability test was used to combine the results over similar types of rivers (I), and Friedman test to analyze the similarity between the naked-eye scores of different observers (IV). In every article, *p*-values from multiple comparisons were Bonferroni-corrected by multiplying them by the number of comparisons, and $p < 0.050$ was considered statistically significant while $0.050 \leq p < 0.100$ was judged as statistically marginally significant.

TABLE 3 The variables compared between different fish groups and the statistical methods used. The first method was always the priority, while the method in parenthesis was used when the requirements of the former test was not met.

| Variable | Definition | Statistical method |
|---|---|---|
| Prevalence of FPM infection | Proportion of fish individuals parasitized by FPM glochidia among all individuals in the sample | $\chi^2$ test (Fisher's exact test) |
| Abundance of FPM infection | Average number of FPM glochidia per fish among all individuals in the sample | ANOVA and Tukey (Mann-Whitney *U* test) |
| Intensity of FPM infection | Average number of FPM glochidia per fish among infected individuals in the sample | ANOVA and Tukey (Mann-Whitney *U* test) |
| Length of FPM glochidia | Longest diameter of round or slightly oval FPM glochidia | ANOVA and Tukey (Mann-Whitney *U* test) |

# 3 RESULTS AND DISCUSSION

## 3.1 Host suitability experiments

### 3.1.1 Differences between Atlantic salmon and brown trout in suitability as *M. margaritifera* hosts (I)

The FPM populations living in the small headwater tributaries of the River Iijoki catchment were found to be generally adapted to use brown trout as their host fish, while Atlantic salmon can be considered a poorer, or even unsuitable, host for these populations. In most tributaries the abundance of FPM glochidia was significantly higher in all of the tested brown trout strains than in salmon (Table 4). Several cases of higher infection prevalence and greater glochidial length in brown trout than in salmon were also found (Table 4). Furthermore, in the laboratory study in which FPM from the Jukuanoja stream was used and which included a monitoring period with 4 examination dates, no glochidia from salmon were found anymore at the third examination point (3 months after the exposure) or later. This indicates premature drop of glochidia and thus Atlantic salmon being completely unsuitable as a host for Jukuanoja FPM.

However, in the Lohijoki stream no suitability differences between salmon and brown trout were found (Table 4), and the results with Koivuoja FPM contradicted the general result whether the suitability test was conducted in the field or in the laboratory; in the caging experiment the glochidia abundance was marginally higher in salmon than in brown trout, and in the laboratory the infectivity of Koivuoja FPM was clearly better in salmon than in brown trout as measured by both prevalence and abundance (Table 4).

The finding that brown trout is usually a better host than salmon in these kinds of small headwater tributaries is not surprising, as brown trout is often the only salmonid species that naturally colonises such streams (Erkinaro 1995, Hastie and Young 2001, Knouft and Spotila 2002), while salmon prefers larger channels (Klemetsen *et al.* 2003, Johansen *et al.* 2005). However, the results underline the importance of the brown trout populations to FPM conservation in small watercourses.

TABLE 4 The differences of various brown trout strains in a comparison with Atlantic salmon (Iijoki strain) in prevalence and abundance of FPM infection and in FPM glochidia length in the experiments in which FPM sourced from small brown trout streams. All the FPM source rivers belong to the River Iijoki catchment. NS = no significant difference.

| Source of FPM | Brown trout strain | Prevalence | Abundance | Glochidia length |
|---|---|---|---|---|
| Lohijoki | Lohijoki | NS | NS | - |
| | Portinjoki | NS | NS | - |
| | Iijoki | NS | NS | - |
| Portinjoki | Portinjoki | NS | NS | - |
| | Lohijoki | NS | sign. higher | - |
| | Iijoki | NS | sign. higher | - |
| Ala-Haapuanoja | Ala-Haapuanoja | NS | sign. higher | NS |
| | Iijoki | NS | sign. higher | NS |
| | Rautalampi | NS | sign. higher | NS |
| Porraslammenoja | Porraslammenoja | NS | marg. higher | NS |
| | Iijoki | sign. higher | sign. higher | NS |
| | Rautalampi | sign. higher | sign. higher | marg. higher |
| Koivuoja (cage) | Iijoki | NS | marg. lower | - |
| Koivuoja (lab) | Iijoki | sign. lower | sign. lower | - |
| Jukuanoja | Iijoki | sign. higher | sign. higher | sign. higher |
| Combined analysis | | sign. higher | sign. higher | sign. higher |

In contrast to the small brown trout streams, the general result was completely different in large river channels, which are either presently (Simojoki) or were before hydropower dam construction (Livojoki, Luttojoki) well-known spawning grounds of Atlantic salmon. In these rivers, salmon is evidently the more appropriate FPM host, as both the prevalence and abundance of FPM infection were significantly higher in salmon than in brown trout in almost all cases (Table 5). In addition, glochidia on salmon were often larger than the conspecifics in brown trout (Table 5). Conspicuously, no single variable was higher in brown trout than in salmon in any experiment or comparison (Table 5).

The adaptation of FPM to salmon in the large channels Luttojoki and Livojoki, the catchments of both of which were dammed over 50 years ago (Karppinen *et al.* 2002, Erkinaro *et al.* 2011), together with the general salmonid stocking practices favouring brown trout as the compensatory species, provides an additional explanation for the collapse of FPM populations, as well as for the lack of successful recruitment of FPM, in large Finnish salmon rivers (Valovirta 1990, Oulasvirta 2011). As there are hardly any functioning fishways in the dams in Finland, the appropriate host, Atlantic salmon, has had no chance to naturally migrate to its original spawning grounds after the dam construction. The lack of the anadromous salmonids, including salmon, has been ordered by the courts to be compensated via annual release of farmed salmonids (e.g. Erkinaro *et al.* 2011), but there have been no strict requirements for the salmonid species used in these stockings. Thus, the former spawning habitats of Atlantic salmon for example in the Iijoki area are now dominated by brown trout, while salmon juveniles have been released only to estuaries near the sea, i.e. below the dams (Hiltunen 2010, Erkinaro *et al.* 2011, Marttila *et al.* 2014). In the Luttojoki area, there have been no regular, annual salmonid introductions. Thus, the disappearance of the appropriate host fish species may even be the main contributor behind the collapse of FPM populations in these rivers, as well as that of many other populations in dammed river systems, especially in Finland where both the collapse of FPM and the intensity of harnessing larger rivers for electricity production have been remarkable.

To my knowledge, the revealed salmon-dependence of certain FPM populations is a unique finding in the Baltic Sea drainage. In Sweden, Österling and Wengström (2015) found only FPM populations which use brown trout as their host, and they also stated that no FPM population in Sweden, i.e. on the western coastline of the Baltic Sea, is known to exclusively parasitize salmon. However, in a Russian river, land-locked salmon from Lake Ladoga (Baltic Sea drainage) was recently found to serve as host for FPM, but the suitability of brown trout as FPM hosts in that river is unknown (Ieshko *et al.* 2016). Outside the Baltic area, Hastie and Young (2001) observed higher infectivity of FPM in salmon than in brown trout in several Scottish rivers, and Karlsson *et al.* (2014) found many Norwegian FPM populations to be exclusively adapted to use only either salmon or brown trout as their host. Thus, my results are consistent with these findings, indicating that the differences between FPM populations in terms of the host specificity may be a universal phenomenon. Therefore, even though loss of host fish has often been suggested as a potential contributor to the collapse of FPM (Young and Williams 1983b, Hastie and Cosgrove 2001, Geist 2010, Makhrov *et al.* 2011, Ostrovsky and Popov 2011, Oulasvirta 2011, Lopes-Lima *et al.* 2016), this host specificity, if occurring also elsewhere, may be an additional, less understood explanation for the collapse, for the absence of young mussels and for the unsuccessful outcomes of the attempts to re-establish the declined FPM populations via host salmonid stocking (Bauer 1988, Valovirta 1990, 1993, Geist *et al.* 2006, Thomas *et al.* 2010, Simon *et al.* 2015). The host specificity should certainly be investigated more comprehensively throughout the range of FPM.

TABLE 5 The differences of various brown trout strains in a comparison with various Atlantic salmon strains in prevalence and abundance of FPM infection and in FPM glochidia length in the experiments in which FPM sourced from large salmon rivers of different catchments. NS = no significant difference.

| Source of FPM | Brown trout strain | Salmon strain | Prevalence | Abundance | Gl. length |
|---|---|---|---|---|---|
| Livojoki (2011) | Iijoki | Iijoki | sign. lower | sign. lower | - |
| | Kitkajoki | Iijoki | sign. lower | sign. lower | - |
| | Rautalampi | Iijoki | sign. lower | NS | - |
| Livojoki (2012) | Iijoki | Iijoki | sign. lower | sign. lower | NS |
| | Kitkajoki | Iijoki | sign. lower | sign. lower | sign. lower |
| | Rautalampi | Iijoki | NS | NS | NS |
| Livojoki (2013) | Iijoki | Iijoki | sign. lower | sign. lower | sign. lower |
| | Iijoki | Simojoki | sign. lower | sign. lower | sign. lower |
| | Iijoki | Tornionjoki | sign. lower | sign. lower | sign. lower |
| Simojoki | Iijoki | Iijoki | sign. lower | sign. lower | NS |
| | Iijoki | Simojoki | sign. lower | sign. lower | NS |
| | Iijoki | Tornionjoki | sign. lower | sign. lower | NS |
| Luttojoki | Iijoki | Iijoki | sign. lower | sign. lower | sign. lower |
| Combined analysis | | sign. lower | sign. lower | sign. lower | sign. lower |

### 3.1.2 Local adaptation of *M. margaritifera* (II)

A tendency was found for higher infectivity of FPM glochidia in local (sympatric) salmonid hosts than in non-local (allopatric) strains of the same host species (Table 6). In brown trout experiments conducted in 4 streams of the River Iijoki catchment and in 2 tributaries of the River Luttojoki, the local brown trout was a better host than either of the non-local strains in 3 streams according to glochidia abundances (Table 6). Significantly higher glochidial length in local brown trout than in non-local was also found in one experiment (Table 6). However, there were no differences in prevalence between the strains, as FPM glochidia were found from every single brown trout from every strain in every experiment.

The results from the salmon experiments also slightly indicated FPM to be locally adapted (Table 6). No differences were found in prevalence or abundance between different salmon strains, but larval length was higher in local salmon than in a non-local salmon in the River Simojoki. Several differences between the non-local strains were also detected (Table 6), but no indication of potential maladaptation of FPM (i.e. fish from any allopatric strain being better hosts than fish from sympatric strain) was found either in the brown trout or in the salmon experiments.

To my knowledge, the study by Österling and Larsen (2013) is the only publication in which the host specificity of FPM between sympatric and allopatric salmonid populations has been investigated earlier. Interestingly, they found non-local brown trout to be better hosts than the local conspecifics. No similar result was obtained in any of our experiments, but as an infection prevalence of 100 % was often detected in non-local host strains in our experiments, too, one can estimate that the potential spread of non-original strains to FPM habitats may not pose a strong threat to FPM. However, for the best success of FPM restoration methods including salmonid stocking (Bauer 1988, Buddensiek 1995, Geist *et al.* 2006, Thomas *et al.* 2010), but also to conserve and restore the natural salmonid populations which often are also declining (Parrish *et al.* 1998, Almodóvar and Nicola 1999, Kallio-Nyberg *et al.* 2001, Burkhardt-Holm *et al.* 2005), stocking only host fish originating from population sympatric with FPM is recommended. Furthermore, results from artificial culture and reintroduction programmes (Buddensiek 1995, Beasley and Roberts 1999, Cosgrove and Hastie 2001, Geist 2010, Thomas *et al.* 2010, Gum *et al.* 2011) may also be enhanced by using the original host strains when available.

TABLE 6 Significant differences in abundance of FPM infection and in FPM glochidia length between different strains of brown trout and Atlantic salmon in the cage experiments. Any differences in prevalence of FPM infection were not found between any strains. Each river experiment included the local strain and two other strains (non-local$_1$, non-local$_2$). NS = no significant difference in any of the comparisons.

| River | Fish species | Abundance | Glochidia length |
|---|---|---|---|
| Ala-Haapuanoja | Brown trout | local > non-local$_1$<br>non-local$_2$ > non-local$_1$ | NS |
| Lohijoki | Brown trout | NS | - |
| Porraslammenoja | Brown trout | non-local$_1$ > non-local$_2$ | NS |
| Portinjoki | Brown trout | NS | - |
| Hanhioja | Brown trout | local > non-local$_1$<br>local > non-local$_2$ | NS |
| Kolmosjoki | Brown trout | local > non-local | local > non-local$_1$<br>non-local$_2$ > non-local$_1$ |
| Livojoki | Salmon | NS | non-local$_1$ > non-local$_2$ |
| Simojoki | Salmon | NS | local > non-local$_1$<br>non-local$_2$ > non-local$_1$ |

### 3.1.3 Suitability of brook trout as a host for the European freshwater pearl mussel (III)

Brook trout cannot be considered a suitable host fish species for FPM populations in the River Iijoki catchment. Using FPM from 4 different origins and several strains of its native European hosts, brown trout and Atlantic salmon, for comparison, both the prevalence and abundance of FPM infection were lowest in brook trout in almost every comparison (Table 7). In one experiment the length of the encysted FPM glochidia was also lower in brook trout than in a comparison fish group, even though the generally low number of glochidia in brook trout rendered the power of statistical tests low. In the second laboratory experiment, which included a monitoring period of 6.5 months, all brook trout lost their FPM glochidia in less than 3 months.

However, exceptionally one indication was found of FPM glochidia metamorphosing potentially successfully to juvenile mussels in brook trout; few wild brook trout individuals captured from the Kostonlammenoja stream were found to harbour FPM glochidia in their gills. Furthermore, as the fish were caught in early June and the size of glochidia had also greatly increased to more than 300 µm from the initial 50–70 µm (e.g. Nezlin *et al.* 1994, Pekkarinen and Valovirta 1996, Denic *et al.* 2015), there is no doubt that the glochidia had been attached to the brook trout for at least 9 months. This case is, to my knowledge, the first ever confirmed successful parasitism by European FPM of this invasive salmonid, and implies that the long-run adaptation of European FPM to use brook trout as their host cannot be excluded. Nevertheless, the prevalence of FPM infection in brook trout in that stream was only 26 %, i.e. as low as the glochidia prevalence in brook trout in the experimental cage and laboratory studies, and no FPM-infected brook trout were found in other FPM streams surveyed.

The general result of this study is in line with earlier research by Bauer (1987c) and Jung *et al.* (2013), who found brook trout to be an inappropriate host for Central European FPM populations in their short-term laboratory experiments. Contradictorily, brook trout is suggested to be a host for FPM in North America (Clarke and Berg 1959, Athearn and Clarke 1962). However, only one, limited, experimental study seems to have been conducted (Smith 1976). In that laboratory study, 2 brook trout individuals were first exposed to high number of FPM glochidia, and both of them suffocated to death in 2 hours, according to the author due to heavy FPM infection. Two other individuals were then infected with moderate glochidial intensity and on inspection after 36 days FPM glochidia were still found attached to their gills. However, no quantitative data about the numbers or the possible growth of glochidia were given. Thus, according to our findings and the other European studies (Bauer 1987c, Jung *et al.* 2013), and the fact that the successful metamorphosing from FPM glochidia to juvenile mussel lasts at least 8 months also in North America (Cunjak and McGladdery 1991), the Smith's (1976) conclusions should be considered preliminary and the suitability of brook trout as an FPM host in North America still awaits confirmation.

In addition to the low suitability of brook trout as a FPM host, it was found that the occurrence of that invader is relatively higher in streams inhabited by FPM and brown trout than in streams with brown trout but no FPM. As costs of FPM infection for the host have been documented (Meyers and Millemann 1977, Taeubert and Geist 2013, Österling *et al.* 2014, Thomas *et al.* 2014), this finding supports the enemy release hypothesis (Sax and Brown 2000, Mitchell and Power 2003) whereby low infectivity by native parasites in invading species may provide a competitive advantage for the aliens over the native species which suffer more intensive parasitism. Thus, the inappropriateness of brook trout as a host for European FPM and its potential to invade FPM rivers and replace the native host of FPM, brown trout, especially in small headwater tributaries of northern Fennoscandia even within 2 decades (Korsu *et al.* 2007, Spens *et al.* 2007, Öhlund *et al.* 2008) makes the spread of brook trout an additional, and previously unrecognized, potential threat to the European FPM. Generally, the dispersion of non-indigenous fishes is considered one of the main threats to biodiversity worldwide and has induced significant global ecological and economic costs, even though it is challenging to accurately determine all their impacts (Pimentel *et al.* 2000, 2001, Clout and Williams 2009, Davis 2009). This kind of consequence – a native bivalve, the reproduction of which depends on a native species, becoming indirectly threatened by an invader which, as a strong competitor, may replace the native host of the parasite – is a representative example of the unexpected results that invasions may induce in aquatic ecosystems. This finding is also another illustration that coextinction in mutualistic or parasitic interactions may be the most common form of extinctions (Dunn *et al.* 2009).

The superiority of brook trout over brown trout had been considered to be the result of characteristics like the faster growth (Öhlund *et al.* 2008, Korsu *et al.* 2009) and the younger maturation age (Öhlund *et al.* 2008) of brook trout, in addition to its ability to spawn also in lakes (Curry *et al.* 1997). A potential new aspect for the interaction between brook trout and the native European salmonids was found in the second laboratory experiment, during of which predation by brook trout on Atlantic salmon was detected in spite of the fact that all fishes were of the same age (0+) and they were fed daily. Furthermore, individuals of brown trout also disappeared due to unknown reason, and Korsu *et al.* (2007) have observed that density of 0+ brown trout reduces in rivers after brook trout invasion. This might indicate predation occurring also in nature, and our observations suggest that another salmonid, Atlantic salmon, can also be vulnerable to displacement after brook trout invasion.

TABLE 7 The differences between brook trout in a comparison with brown trout and Atlantic salmon in prevalence and abundance of FPM infection and in FPM glochidia length with different sources of FPM. All the FPM source rivers belong to the River Iijoki catchment. The subscripted numbers separate different strains of brown trout. NS = no significant difference.

| Source of FPM | Species | Prevalence | Abundance | Glochidia length |
|---|---|---|---|---|
| Koivuoja | Brown trout $_1$ | sign. lower | sign. lower | - |
| | Brown trout $_2$ | sign. lower | sign. lower | - |
| | Brown trout $_3$ | sign. lower | sign. lower | - |
| | Brown trout $_4$ | NS | NS | - |
| | Atlantic salmon | marg. lower | marg. lower | - |
| Jukuanoja | Brown trout | sign. lower | sign. lower | NS |
| | Atlantic salmon | sign. lower / NS | sign. lower / NS | NS |
| Porraslammenoja | Brown trout $_1$ | sign. lower | sign. lower | NS |
| | Brown trout $_2$ | sign. lower | sign. lower | sign. lower |
| | Brown trout $_3$ | sign. lower | sign. lower | NS |
| | Atlantic salmon | sign. lower | sign. lower | NS |
| Kostonlammenoja | Brown trout | NS | NS | NS |

## 3.2 Electrofishing to search for *M. margaritifera* populations (IV)

The results from both the field and laboratory clearly indicate that electrofishing, a standard method for catching juvenile salmonids non-destructively (Hudy 1985, Bohlin *et al.* 1989), followed by a quick, non-destructive naked-eye examination of gills of captured salmonids still stunned in consequence of the electric shock, can be reliably used for mapping the occurrence of FPM. Examination of salmonid samples, first with naked eyes and then microscopically, collected from 40 previously unsurveyed or inadequately surveyed tributaries of the River Iijoki catchment revealed the occurrence of FPM in 3 rivers. Most importantly, in each case the naked-eye examination identified the occurrence of FPM glochidia in salmonids captured from these rivers.

Before applying the method in practice, the accuracy of the naked-eye examination was tested in 4 previously confirmed FPM rivers. The infection status of fish (infected or uninfected by FPM) was correctly identified in 62–93 % of fish captured in these rivers in June–July, when the glochidia had been on the hosts over a winter and had greatly increased in size. In the laboratory experiment with artificially infected fish, the accuracy of the status assessment was 96 %. Furthermore, in each of these field and laboratory samples the accuracy of the identification was 100 % whenever there were more than 20 glochidia individual per fish. However, the naked-eye examination was not reliable in autumn, shortly after the attachment of the new glochidium generation, due to the small size of glochidia at that time.

The large glochidia also enabled successful estimation of the intensity of infection, as the mean of the naked-eye scores, from 0 (no glochidia) to 5 (very high number of glochidia), assessed by 2 or 3 persons always correlated significantly with the actual number of glochidia confirmed microscopically. Furthermore, both fish-level and river-level mean scores of higher than 0.5 identified the true FPM occurrence with 100 % reliability. The scores of the independent observers also correlated significantly to each other.

Since many freshwater mussels, including FPM, have declined (e.g. Bauer 1988, Williams *et al.* 1993, Lydeard *et al.* 2004), one challenge for conservation of the remaining populations is to find as many as possible of the still unknown mussel beds. In a small river with clear water, one can perhaps find a mussel without any device. However, detecting a mussel is often difficult even in a habitat in which its occurrence is confirmed, and factors like dark and turbid water may further impede the visual finding of mussels (Young and Williams 1983a, Álvarez-Claudio *et al.* 2000, Cosgrove *et al.* 2000, Oulasvirta 2011). A large river area, in addition to a large number of small brooks, as is typical of catchments e.g. in northern Finland, may also limit the use of the traditional visual search methods. However, with the presented electrofishing method, one can obtain an indication of the occurrence of FPM in a river with a quick catch of probably only one salmonid, if it is parasitized by FPM glochidia. Thus, in certain circumstances this new approach may prove to be faster and easier way to detect mussels than haphazard diving or aquascope searching; our field survey included electrofishing in 40 rivers, some of them twice and in several sections, and took less than 4 weeks. The advantages of the method include the simultaneous acquisition of information about the occurrence and the diversity of fish species in the surveyed rivers. Furthermore, as electrofishing is generally non-destructive for both fish (Hudy 1985, Bohlin *et al.* 1989) and mussels (Hastie and Boon, 2001), there are no ethical restrictions on using this method even in the smallest streams with potentially low fish and mussel density. However, it should be noted that the occurrence of adult mussels must also be confirmed when glochidia in salmonids are found, because possible migrations of FPM-parasitized salmonids may confuse the findings, despite the fact that a resident brown trout usually stays within a small area throughout the year (e.g. Burrell *et al.* 2000, Knouft and Spotila, 2002). As an additional limitation, the electrofishing method including naked-eye examination of FPM glochidiosis should only be used in spring or early summer, i.e. when the glochidia are large enough to be visible by eye but not yet detached. For detection of freshly attached glochidia in autumn, one should probably use a photo-method, another non-destructive technique presented recently by Österling (2011).

# 4 CONCLUSIONS

Damming of rivers for hydropower production was very intensive in Finland during the 20th century. The consequent disappearance of the original populations of anadromous salmonids was compensated by well-intended but, in terms of natural between-species interactions, inadequately designed salmonid stockings, which have caused for example the colonization of the River Iijoki drainage by brook trout. In addition, brown trout, often from non-original strains, have been stocked in the former spawning grounds of Atlantic salmon, whereas salmon juveniles have been released only in the river mouths below the dams. The results of this thesis imply that both the replacement of salmon by brown trout as well as the spread of brook trout may have contributed to the collapse of freshwater pearl mussel (FPM). Thus, conservation of FPM requires changes to these common stocking-based management practices; brook trout should be removed rather than stocked in the future and, most importantly, Atlantic salmon should be restored to its former habitats where FPM has suffered from lack of the most appropriate host for many decades. Due to the high and lifelong fecundity of this extremely long-lived mussel, there is still potential to restore the reproduction of FPM and thus enable the recovery of FPM in these dammed river systems.

In contrast to stocking of salmonid species alien to a particular area, stocking of fish from the appropriate host species but from a non-original strain may not be harmful for FPM; although several pieces of evidence were found to support local adaptation, i.e. the theory that parasites infect better sympatric than allopatric hosts, even brown trout originating from non-FPM catchments far from the study rivers were successfully parasitized by FPM in the experiments. However, as no specific reasons to use non-original strains in terms of FPM success or river ecosystem function are known, using the local fish strain would appear to be the best practice in salmonid stocking in any case. The slightly better FPM infectivity in sympatric salmonid strains further suggest that using the original host strain is also advisable to produce the maximum number of juvenile mussels artificially in captive breeding programs (Buddensiek 1995, Thomas *et al.* 2010, Gum *et al.* 2011).

Despite the long history of FPM research, previously unknown populations have occasionally been found even in the 21th century, indicating that the geographical distribution of FPM is not completely mapped. The 3 FPM populations found in the River Iijoki drainage in the present study can now be added to the list of the recently found populations. However, the way the populations were detected in the present study was completely different from the previous approaches; this method, herein proven to be functional, was based on quick catch and non-destructive but accurate examination of the potential host species of FPM. This approach may provide the most effective method to search for FPM in dark and turbid waters over large areas, i.e. in the rivers where visual detection of adult mussels is time-consuming. Furthermore, this method may be used to search for other mussel species too, and the naked-eye examination can be easily added to other electrofishing work conducted in unexplored, potential salmonid rivers; in these days of limited research funding, piggybacking of two separate surveys may give an additional value to the work. However, neither this nor the traditional methods can be used to confirm the absence of FPM from a river.

The thesis shows more widely the importance of taking into account the relationship between FPM and the potential salmonid host species in the context of future conservations actions; the salmonid used in fish stocking to FPM habitats has to be the original salmonid species of the area, and no invasive fish species should be introduced. Furthermore, the new electrofishing method demonstrates the potential for utilizing salmonid hosts to map the occurrence of freshwater pearl mussels.

*Acknowledgements*

I am grateful to the Maj and Tor Nessling Foundation for their one-year financial grant, which initiated this PhD project in 2011. After the first year, I was privileged to be admitted as a member of the Doctoral Programme in Biological and Environmental Science of the University of Jyväskylä, which also included three-year financial support for this project. Later, grants from the OLVI Foundation and the Finnish Foundation for Nature Conservation enabled finalizing of the thesis. Project "Raakku!" (funded by EU Interreg IV A Nord Programme and granted to Jouni Taskinen) provided the required financial resources to cover the research costs such as equipment, travelling and accommodation during my doctoral studies. The project "Raakku!", as well as a travel grant by the Malacological Society of London, also enabled me to present my results in seminars in Bragança, Portugal (2012), Kefermarkt, Austria (2013), Rovaniemi, Finland (2014) and Buffalo, USA (2015), and thus to network internationally with other mussel researchers.

On the personal level, I first thank deeply my supervisors, Prof. Jouni Taskinen and Dr. Timo J. Marjomäki, for their endless support during these years. I also express my warmest gratitude to Pirkko-Liisa Luhta and Eero Moilanen whose roles to execute experiments and surveys in the field were indispensable. Laboratory technician Olli Nousiainen also deserves my special thank for his tremendous effort in field work, and "salmonid specialist" Jukka Syrjänen for helping me especially in preparing the manuscripts. The members of the follow-up group, Heikki Hämäläinen and Jukka Syrjänen, encouraged me throughout my doctoral studies. I also thank the project "Raakku!" coordinator, Panu Oulasvirta, for the possibility of co-operation. I also acknowledge Matti Mela and Markku Seppänen for their support in the field, and Prof. Roger Jones, Prof. Dale Edwards, Dr. Peter Cosgrove and Dr. Jocelyn Mah Choo for English language checking and commenting on this thesis and the manuscripts. Many other people also used their time to comment on my manuscripts during these years; many thanks to all of you.

For successful laboratory studies in the Konnevesi Research Station, I thank the technical staff Janne Koskinen, Risto Latvanen and Jyrki Raatikainen for taking care of my fish and mussels throughout this project. The technicians in the Department of Biological and Environmental Science (Juha Ahonen, Ahti Karusalmi and Hannu Kautto) also deserve my gratitude.

Last but not least, I acknowledge the other "*Margaritifera* students" – Motiur Chowdhury, Felix Luukkanen, Tapani Säkkinen, Jarno Turunen and Santtu Välilä – in the University of Jyväskylä for their help, support and friendship. Thanks also for other friends for counterbalancing the academic work with recreational activities outside the University.

# YHTEENVETO (RÉSUMÉ IN FINNISH)

## Lohikalojen rooli jokihelmisimpukan (*Margaritifera margaritifera*) suojelussa

Jokihelmisimpukka (*Margaritifera margaritifera*), kansankielellä raakku, elää puhtaissa, usein luonnontilaisina säilyneissä Atlantin valtamereen laskevissa virtavesissä pohjoisella pallonpuoliskolla. Laji on taantunut merkittävästi viimeisen sadan vuoden aikana; eurooppalaisista jokihelmisimpukkapopulaatioista on kadonnut jopa 95 %, minkä lisäksi suuressa osassa jäljellä olevista populaatioista lisääntyminen on loppunut lähes kokonaan. Suomessakin oli vielä 1900-luvun alussa ainakin 200 raakkujokea, mutta nykyään lisääntyvä kanta löytyy tiettävästi enää vain muutamasta kymmenestä joesta. Pääsyynä taantumiseen pidetään teollistumisen ja muun ihmisperäisen toiminnan myötä vesistöissä tapahtuneita muutoksia. Esimerkiksi Suomessa lähes kaikki suuret joet ruopattiin ja perattiin tukinuittoa varten 1900-luvun alkupuolella; samalla tuhoutui lukuisia simpukkapopulaatioita ja niiden elinalueita. Tukinuittoaikakauden jälkeen vesivoimarakentaminen on muokannut virtavesiä voimakkaasti, sillä 1960-luvulla lähes kaikki suuret lohijoet padottiin vesivoiman tuotantoon, mikä esti raakun lisääntymisessään tarvitsemien lohikalojen pääsyn jokiin. Myöhemmin esimerkiksi metsäteollisuuden ja -ojitusten virtavesiin laskemat päästöt ovat olleet haitallisia etenkin nuorille simpukoille, jotka eivät kestä äkkinäisiä olosuhdemuutoksia yhtä hyvin kuin aikuiset yksilöt. Eittämättä myös Suomessa jo vuonna 1955 kielletyllä helmenpyynnillä on ollut oma roolinsa jokihelmisimpukan taantumisessa.

Jokihelmisimpukan suojelua vaikeuttaa lajin monimutkainen, mutta simpukoille yleisesti luonteenomainen elinkierto. Eurooppalainen raakku "kutee" loppukesällä, jolloin koiraat vapauttavat siittiönsä veteen. Naarassimpukat ottavat siittiöt virrasta suodattamansa veden mukana sisäänsä, jolloin niiden munasolut hedelmöittyvät. Myöhemmin alkusyksystä naaraat vapauttavat hedelmöittyneet, n. 70 µm pituiset ns. glokidium-toukat veteen, jolloin toukkien tulee löytää isäntäkala, joksi nykytietämyksen mukaan soveltuu vain joko lohi (*Salmo salar*) tai taimen (*S. trutta*). Onnistuneesti isäntäkalan kiduksille tarttuneet toukat elävät kalassa loisina lähes vuoden; Pohjois-Euroopassa loisintavaihe päättyy tarttumisajankohtaa seuraavan kesän loppupuolella, jolloin toukat irrottautuvat kaloista noin 0,5 mm mittaisina pikkusimpukoina. Sekä glokidiumtoukkien että nuorten simpukoiden kuolleisuus on suurta, mutta näistä ns. pullonkaulavaiheista selviydyttyään jokihelmisimpukka voi elää jopa yli 200-vuotiaaksi.

Huolimatta lisääntyneestä tutkimustiedosta, jokihelmisimpukan taantumisen ja siihen johtaneiden syiden tiedostamisesta sekä monista raakkujokien ja -populaatioiden ennallistamiseen tähtäävistä toimista tämän uhanalaiseksi luokitellun lajin tilanne ei ole juurikaan parantunut. Täten onkin todennäköistä, että kaikkia syitä populaatioiden häviämisiin ei ole vielä tunnistettu. Tässä väitöstutkimuksessa näitä lisäsyitä etsittiin selvittämällä eri raakkupopulaatioiden mahdollista isäntäspesifisyyttä simpukan tunnettujen isäntäkalalajien, lohen ja taimenen (I), ja näiden eri kantojen (II), välillä. Osittain samoissa kokeissa tutkittiin myös (III), kykeneekö eurooppalainen raakku käyttämään isäntänään tulokaslaji puronieriää (*Salvelinus fontinalis*), joka on voimakkaana kilpailijana syrjäyttänyt alkuperäisen eurooppalaisen lohikalan ja raakun isännän, taimenen, monissa puroissa. Lopuksi esitellään uudenlainen menetelmä vielä löytämättömien raakkupopulaatioiden etsintään (IV).

Isäntäspesifisyyttä (I–III) tutkittiin altistamalla kaloja raakkuinfektiolle sumputtamalla niitä raakkujoissa ja -puroissa simpukoiden glokidiumparveilun aikaan syksyllä sekä altistamalla kaloja keinotekoisesti raakun glokidium-toukille laboratoriossa. Kalojen kiduksille tarttuneiden toukkien määrä laskettiin ja niiden pituus mitattiin kokeesta riippuen 1–6 kk raakkualtistuksen jälkeen, minkä jälkeen mahdolliset erot infektoituneiden kalojen osuudessa, tarttuneiden toukkien lukumäärässä sekä toukkien pituudessa tutkimuskalaryhmien välillä analysoitiin. Eri raakkupopulaatioiden isäntäspesifisyydessä lohen ja taimenen välillä (I) havaittiin selvät erot sekä Iijoen, Simojoen että Tuulomajoen vesistöalueilla. Atlantin lohen entisillä tai nykyisillä nousualueilla (Livojoen, Simojoen ja Luttojoen pääuomat) elävien raakkujen glokidiumtoukat tarttuivat selvästi paremmin loheen kuin taimeneen. Sen sijaan Iijoen vesistön pienemmillä purotaimenten asuttamilla sivupuroilla, joihin lohi ei tiettävästi ole koskaan noussut, taimen oli yleisesti lohta soveliaampi tai jopa ainoa sovelias isäntäkala raakulle. Muutamasta pienemmästä purosta löytyi kuitenkin myös raakkupopulaatio, joka pystyi käyttämään isäntänään yhtä hyvin sekä lohta että taimenta, minkä lisäksi yhdessä sivupurossa raakuntoukat tarttuivat paremmin loheen kuin taimeneen.

Jokihelmisimpukan mahdollisesta sopeutumisesta oman kotivesistön paikalliseen isäntäkalapopulaatioon (II), saatiin jonkin verran näyttöä. Niin taimen- kuin lohikokeissakin eri raakkupopulaatioiden glokidium-toukat tarttuivat runsaslukuisesti sekä paikallisen kannan kaloihin että vieraiden, eipaikallisten kantojen kaloihin, mutta varsinkin Tuuloman alueen kahdessa sivupurossa tarttuneiden toukkien määrä ja paikoitellen kokokin oli suurempi paikallisissa taimenissa kuin vieraiden kantojen taimenissa.

Puronieriän havaittiin olevan selvästi raakun alkuperäisiä eurooppalaisia isäntälajeja, lohta ja taimenta, huonompi isäntä (III). Kaikki kokeet huomioiden glokidium-toukkia löytyi vain harvemmasta kuin joka neljännestä puronieriästä, kun esiintyvyys parhaassa isäntälajissa oli lähes aina 100 %. Myös tarttuneiden toukkien keskipituus oli puronieriässä usein pienempi kuin vertailukaloissa. Lisäksi yli puolen vuoden pituisen seurantajakson sisältäneessä laboratoriokokeessa puronieriöihin tarttuneet toukat irtosivat kaloista jo 3 kk sisällä altistuksesta. Kokeellisten altistusten lisäksi puronieriän luonnollista altistumista raakulle tutkittiin, ja eräästä raakkujoesta pyydetyistä 5 todennäköisesti luonnossa syntyneestä puronieriäyksilöistä löytyi pieni määrä glokidium-toukkia, jotka olivat loisineet kaloissa vähintään 9 kk ajan ja myös kasvaneet loisinnan aikana. Loisittujen kalojen osuus sekä toukkien lukumäärä ja niiden pituus olivat puronieriöissä pienemmät kuin samasta joesta pyydetyissä taimenissa, mutta havainto jättää avoimia kysymyksiä raakun mahdollisesta evolutiivisesta kyvystä sopeutua käyttämään isäntäkalanaan myös puronieriää. Yleisesti tämän työn tulosten perusteella puronieriää on kuitenkin pidettävä uhkana paitsi alkuperäisille eurooppalaisille lohikaloille, myös näiden lajien olemassaolosta suoraan riippuvaiselle jokihelmisimpukalle. Selvitys puronieriän levinneisyydestä Iijoen alueelle antoi vieläpä viitteitä siitä, että puronieriä yleistyy jatkossa erityisesti juuri raakkupuroissa; puronieriä saattaa hyötyä raakun loisinnan isäntäkaloilleen aiheuttamista kustannuksista, joten se voi menestyä elintila- ja ravintokilpailussa raakkuinfektion heikentämää taimenta vastaan paremmin suhteessa infektoitumattomaan taimeneen.

Esimerkiksi Pohjois-Suomen erämaissa on paljon pieniä, potentiaalisia raakkujokia ja -puroja, joiden raakkuesiintyminen kartoittaminen sukeltamalla tai vesikiikareilla vaatii runsaasti aikaa ja muita resursseja. Osajulkaisussa VI esitellään uudenlainen, tietyissä olosuhteissa mahdollisesti nopeampi ja vaivattomampi menetelmä simpukkapopulaatioiden etsintään; keväällä–alkukesällä, jolloin raakuntoukat ovat loisineet kaloissa 8–10 kk ajan ja kasvaneet sinä aikana riittävän suuriksi, pystytään toukkien esiintyminen kalan kiduksilla havaitsemaan paljain silmin, tappamatta kalaa ja toukkia. Menetelmää testattiin ensin tunnetuissa raakkupuroissa ja myöhemmin laboratoriossa keinotekoisesti infektoiduilla kaloilla, minkä jälkeen sitä käytettiin Iijoen vesistöalueella sähkökalastamalla lohikaloja yhteensä 40 aiemmin tutkimattomasta, potentiaalisesta jokihelmisimpukkajoesta tai -purosta. Raakuntoukilla infektoituneita taimenia saatiin yhteensä 3 purosta. Loisinta havaittiin näissä tapauksissa paljain silmin, ja myös aikuisten simpukoiden esiintyminen kyseisissä puroissa varmistettiin myöhemmin. Näin ollen jo menetelmän ensikokeilu paljasti 3 aiemmin tuntematonta jokihelmisimpukkapopulaatiota.

Tutkimus antoi kokonaisuudessaan runsaasti uudenlaista näkökulmaa uhanalaisen jokihelmisimpukan suojeluun. Erityisesti tiettyjen raakkupopulaatioiden sopeutuneisuus käyttämään isäntään vain joko lohta tai taimenta on tärkeä havainto, joka tulee ottaa huomioon suojelutoimenpiteitä suunniteltaessa. Erityisesti Suomessa vesivoimarakentaminen on 1950-luvulta lähtien estänyt lohen nousun lähes kaikkiin sen entisiin kutujokiin, kuten tässä tutkimuksessa mukana olleisiin Livojokeen ja Luttojokeen, joiden raakkupopulaatiot osoittautuvat selvästi lohesta riippuvaisiksi. Myös patoamattomana säilyneen Simojoen raakkupopulaatio on selvästi sopeutunut käyttämään isäntänään taimenen sijaan lohta. Koska toimivia kalateitä ei esimerkiksi Iijoen voimalaitosten padoissa ole lainkaan ja koska sähköyhtiöiden vuosittaiset velvoiteistutukset on toteutettu istuttamalla alueelle lohen sijaan paitsi taimenta myös invaasiolaji puronieriää, on ilmiselvää, että oikean isäntäkalalajin, lohen, puuttuminen on ollut myötävaikuttamassa entisten lohennousualueiden raakkupopulaatioiden taantumiseen. On myös selvää, että mikäli lohta ei tavalla tai toisella palauteta näille alueille, kuten Livojokeen tai Luttojokeen, kyseisten jokien raakkupopulaatiot kuolevat sukupuuttoon lähitulevaisuudessa. Raakun pitkäikäisyys sekä sen läpi koko elämän kestävä lisääntymiskyky huomioiden näidenkin populaatioiden pelastaminen on kuitenkin vielä mahdollista. Myös puronieriän soveltumattomuus raakun isäntäkalaksi tulee huomioida raakun suojelussa: puronieriää ei pidä istuttaa etenkään raakkualueille, sillä voimakkaana kilpailija se saattaa hävittää alkuperäisen lohikalan ja raakun tarvitseman isäntäkalan, taimenen, vieden samalla raakulta lisääntymismahdollisuuden. Väitöskirjatyössäni kehittämääni uutta sähkökalastusmenetelmää raakkupopulaatioiden etsintään pystytään myös hyödyntämään raakun suojelussa. Menetelmä on monissa olosuhteissa sukeltamista nopeampi tapa hankkia viitteitä raakun esiintymisestä. Lisäksi monet kalastotutkimukset toteutetaan sähkökalastamalla, joten potentiaalisia raakkualueita tutkittaessa kalastuksiin voitaisiin helposti sisällyttää lohikalojen kidusten silmämääräinen analysointi ennen kalojen vapauttamista. Tällöin jokihelmisimpukan esiintymisestä voitaisiin saada tietoa myös muiden kuin erillisten simpukkatutkimusten yhteydessä ja mahdollisesti löydetyt populaatiot saataisiin suojelun piiriin nopeammin.

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# ORIGINAL PAPERS

# I

## ATLANTIC SALMON (*SALMO SALAR*) AND BROWN TROUT (*SALMO TRUTTA*) DIFFER IN THEIR SUITABILITY AS A HOST FOR THE ENDANGERED FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA*) IN NORTHERN FENNOSCANDIAN RIVERS

by

Jouni K. Salonen, Pirkko-Liisa Luhta, Eero Moilanen, Panu Oulasvirta, Jarno Turunen & Jouni Taskinen 2016

Submitted manuscript

# II

# ORIGIN MATTERS: FRESHWATER PEARL MUSSELS SHOW ADAPTATION TO SYMPATRIC SALMONID HOST STRAINS

by

Jouni Taskinen & Jouni K. Salonen 2016

Manuscript

III

# AN ALIEN FISH THREATENS AN ENDANGERED PARASITIC BIVALVE: THE RELATIONSHIP BETWEEN BROOK TROUT (*SALVELINUS FONTINALIS*) AND FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA*) IN NORTHERN EUROPE

by

Jouni K. Salonen, Timo J. Marjomäki & Jouni Taskinen 2016

Aquatic Conservation: Marine and Freshwater Ecosystems
doi: 10.1002/aqc.2614.

# IV

## ELECTROFISHING AS A NEW METHOD TO SEARCH FOR UNKNOWN POPULATIONS OF THE ENDANGERED FRESHWATER PEARL MUSSEL *MARGARITIFERA MARGARITIFERA*

by

Jouni K. Salonen & Jouni Taskinen 2016

Aquatic Conservation: Marine and Freshwater Ecosystems.
In press.