LIFE ReBorN – Evaluation of prevalence and intensity of glochidia on Salmonidae

2017 - 2021

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Introduction

During the LIFE-project ReBorN (LIFE15 NAT/SE/000892) several actions have been made to enhance the habitat for aquatic organisms. The goal was to restore at least 200 km of streams and to build 2300 spawning sites for the Atlantic salmon *Salmo salar*. Now when the project is over and the results are summarized the total length of restored streams are 255 km, and >14 000 spawning sites have been built. Target species in this project were Atlantic salmon, freshwater pearl mussel (FPM) *Margaritifera margaritifera*, and otter *Lutra lutra*. This report concentrates on the FPM.

The FPM have an obligate simple parasitic stage in their life cycle. The life cycle includes an egg stage, larvae (glochidia) stage, juvenile stage, and the adult stage. It is the larvae stage that is parasitic. The glochidia attach to the gills of their host in the late summer (August to September) and it takes approximately 10 months to fulfill the metamorphose (Taeubert et al., 2013).

The FPM is a fish host specialist meaning it can only metamorphose to a juvenile mussel on Atlantic salmon and brown trout *S. trutta*. The general assumption is that young of the year fish is better as host since they have never been infected by glochidia and therefore should not have a required immune system for the parasite. In Central Europe the most used fish host is brown trout but in Scotland and Norway many populations of FPM are supported by Atlantic salmon. In Sweden, the knowledge about Atlantic Salmon as fish host to the FPM is scarce.

In this report, the results from investigations in the LIFE ReBorN project of prevalence (number of infected fish in a population) and infection intensity (number of glochidia on the individual fish) from freshwater pearl mussel glochidia before and after restoration will be summarized. The results can be used as base line measurements for future investigations. The overall hypothesis is that there will be an increase in prevalence (number of fish host infected) and infection intensity (number of glochidia on the infected host) after stream restorations caused by an increase of in-stream residence time that will happen when the habitat is improved.

Material and methods

In the LIFE ReBorN project 14 streams have been investigated for prevalence and intensity (Table 1).

Table 1. The names of the streams, number of years investigated, and number of sites investigated.

River	No years (N)	Sites (N)
Abramsån	1	4
Bladtjärnbäcken	3	1
Blåbergsjöbäcken	3	2
Blåtjärnbäcken	3	1
Forsträskån	1	1
Holmsjöbäcken	3	2
Karlsbäcken	3	3
Långträskälven	1	2
Lögdeälven	3	7
Mjösjöån	3	1
Rutnajoki	2	4
Råneälven	3	4
Vitbäcken	3	5
Åbyälven	3	4

All streams have been investigated in May or June between 1 to 3 times and at 1 to 7 sites (Table 1). To collect fish for the investigation a qualitative electrofishing was conducted in every stream and at multiple sites (Table 1) (Degerman & Sers, 2017).

Prevalence was calculated as the number of infected fish divided by the total amount of fish caught in a stream or at one site. For most streams data was pooled together from all sites.

Intensity was investigated in the field. If there were more than 50 or 100 glochidia on the fish, the count was set to >50 and >100. In the calculations these measurements were set to 50 and 100.

The intensity can also be classified in a five-degree scale 0-4; 0=0 larvae, 1=1-10 larvae, 2=11-50 larvae, 3=51-100 and 4=>100 larvae. The classification can then be used to calculate a weighted mean value for each site. The weighted mean value can have a value between 0-4 and is calculated as follows: number of fishes per class multiplied with the class point number, the value is summarized and divided with the total number of fishes caught.

For differences in prevalence in the streams and between years a Chi 2 test was used. Significance level was set to $p < .05$.

For differences in intensity between fish species, streams, and years a Mann Whitney U-test was used. Significance level was set to $p < .05$. Sample size must be greater than 5 to perform the test.

The length of fish is presented in mm and as the average length of *N* individuals ± the 95% confidence interval.

Results

In total, 14 streams and 39 sites have been investigated between 2017 and 2021. Three streams (21%) have been fished once, one stream (7%) twice and ten streams (72%) have been fished three times. Glochidia infected fish was found at 26 sites (66%) (Table 2). In total, 1239 fishes have been investigated, 62% Atlantic salmon and 38% brown trout. Salmon were more likely than trout to be infected by glochidia (Figure 1). A chi-square test of independence was performed to examine the relation between species and the prevalence of infection. The relation between these variables was significant, X^2 (1, N = 1239) = 44.7, p < .00001).

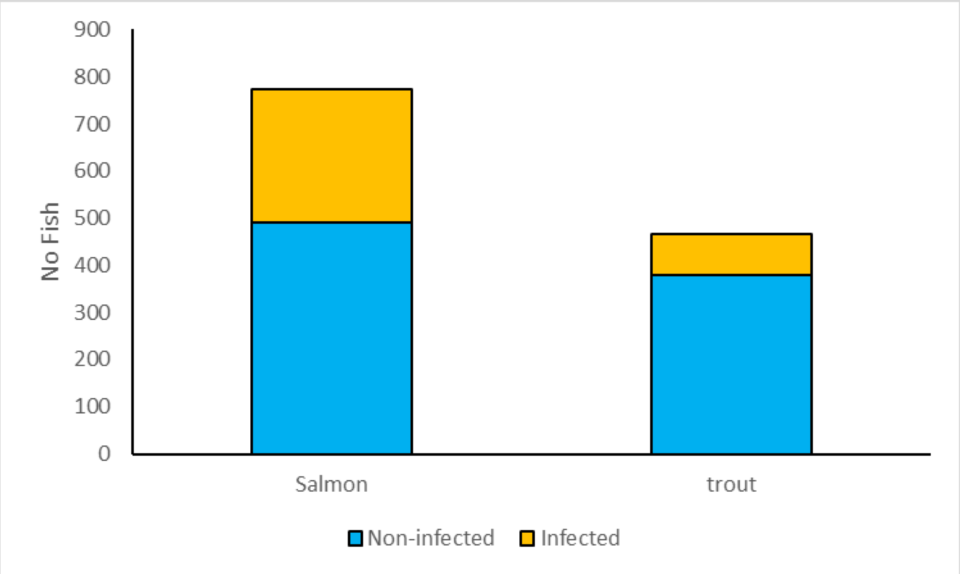

Figure 1. A comparison between Atlantic salmon and brown trout and the number of fish being non-infected and infected by freshwater pearl mussel glochidia.

The average length on infected and non-infected fish differed between species (Figure 2). A Mann-Whitney U test show a significant difference in length between non-infected and infected salmon (Z = 4.8, p < .00001). The difference in length between non-infected and infected brown trout was not significant (p = .24) (Figure 2).

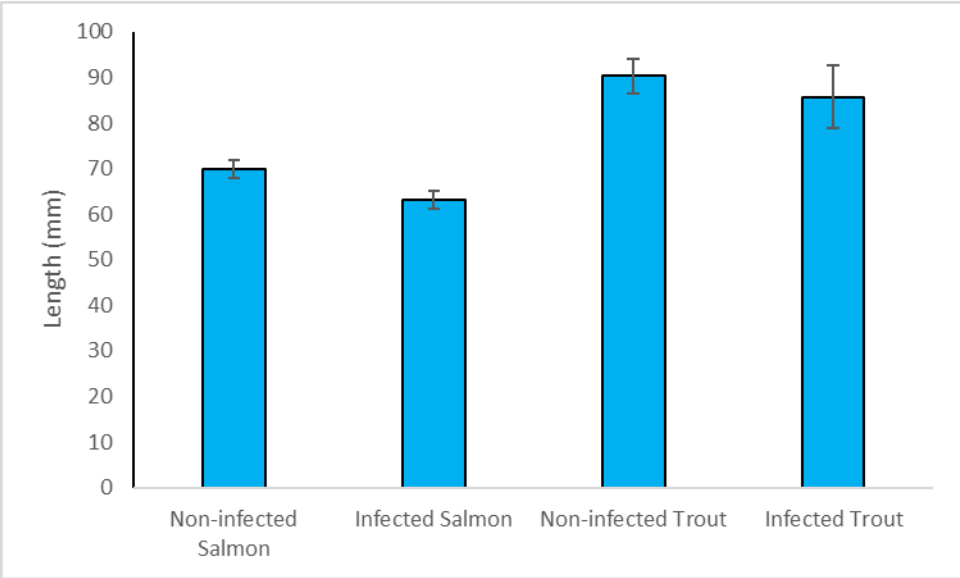

Figure 2. A comparison of length between non-infected and infected salmon and trout. Error bars showing a 95% confidence interval.

Table 2. The names of the streams, number of sites electrofished and sites with infected fish hosts, prevalence and average intensity, fish host species and investigation year.

Stream name	Sites (N)	Sites with infection (%)	Prevalence (%)	Average intensity (N)	Fish Host	Investigation year
Abramsån	4	50	75	7.33	Brown trout	2017
Bladtjärnbäcken	1	100	10	1	Brown trout	2017
Bladtjärnbäcken	1	100	18	7	Atlan-tic salmon	2020
Bladtjärnbäcken	1	0	0	0	-	2021
Blåbergsjöbäcken	2	100	67	61	Brown trout	2017
Blåbergsjöbäcken	2	50	29	25.5	Brown trout	2020
Blåbergsjöbäcken	2	50	69	98.3	Brown trout	2021
Blåtjärnbäcken	1	0	0	0	-	2017
Blåtjärnbäcken	1	0	0	0	-	2020
Blåtjärnbäcken	1	100	100	1	Brown trout	2021
Forsträskån	2	0	0	0	-	2017
Holmsjöbäcken	2	100	10	1.2	Brown trout	2020
Holmsjöbäcken	2	2	23	1.7	Brown trout	2021
Karlsbäcken	3	33	10	1	Brown trout	2020
Karlsbäcken	3	67	16	9	Brown trout	2021
Långträskälven	1	0	0	0	-	2017
Lögdeälven	7	71	15	1.7	Brown trout	2017
Lögdeälven	7	57	7	1	Brown trout	2020

Lögdeälven	7	57	12	1	Brown trout	2021
Lögdeälven	7	86	42	14.8	Atlan-tic salmon	2017
Lögdeälven	7	71	48	11	Atlan-tic salmon	2020
Lögdeälven	5	57	7	11.6	Atlan-tic salmon	2021
Mjösjöån	1	0	0	0	-	2017
Mjösjöån	1	0	0	0	-	2020
Mjösjöån	1	0	0	0	-	2021
Rutnajoki	4	0	0	0	-	2020
Rutnajoki	4	25	50	75	Brown trout	2021
Råneälven	4	75	81	10	Atlan-tic salmon	2017
Råneälven	4	100	92	14.1	Atlan-tic salmon	2020
Råneälven	4	100	91	9.7	Atlan-tic salmon	2021
Vitbäcken	5	60	27	7.3	Brown trout	2017
Vitbäcken	5	20	25	1	Brown trout	2020
Vitbäcken	5	20	33	2	Brown trout	2021
Åbyälven	4	25	25	1.3	Atlan-tic salmon	2017
Åbyälven	4	0	0	0	-	2020
Åbyälven	4	50	46	1.2	Atlan-tic salmon	2021

Abramsån

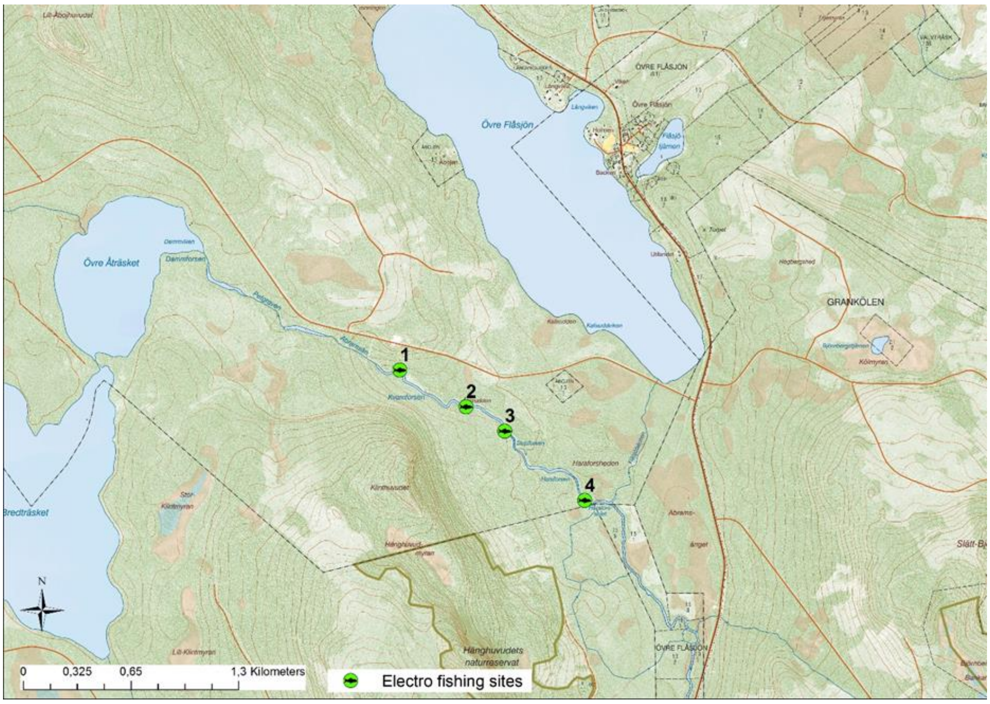

Figure 3. Location of the four different electro fishing sites in River Abramsån. © Lantmäteriet.

In 2017 four sites in the stream was investigated (Figure 3). Glochidia infected trout was found at two of the sites. The prevalence of infected brown trout was 75% (n=4). The average length of the infected trout (n = 3) was 88.7 mm ± 11.2, the non-infected trout (n = 1) was 85 mm. The infection intensity was on average 7.3 glochidia per fish and the range was the between 3-13 glochidia.

The FPM populations in Abramsån consist of a few older individuals and it is at risk of being extirpated (Olofsson, 2018). It probably needs further investigations as two juveniles was found in 2013.

No habitat improvements have been performed at any of the sites where the fish was caught.

Forsträskån

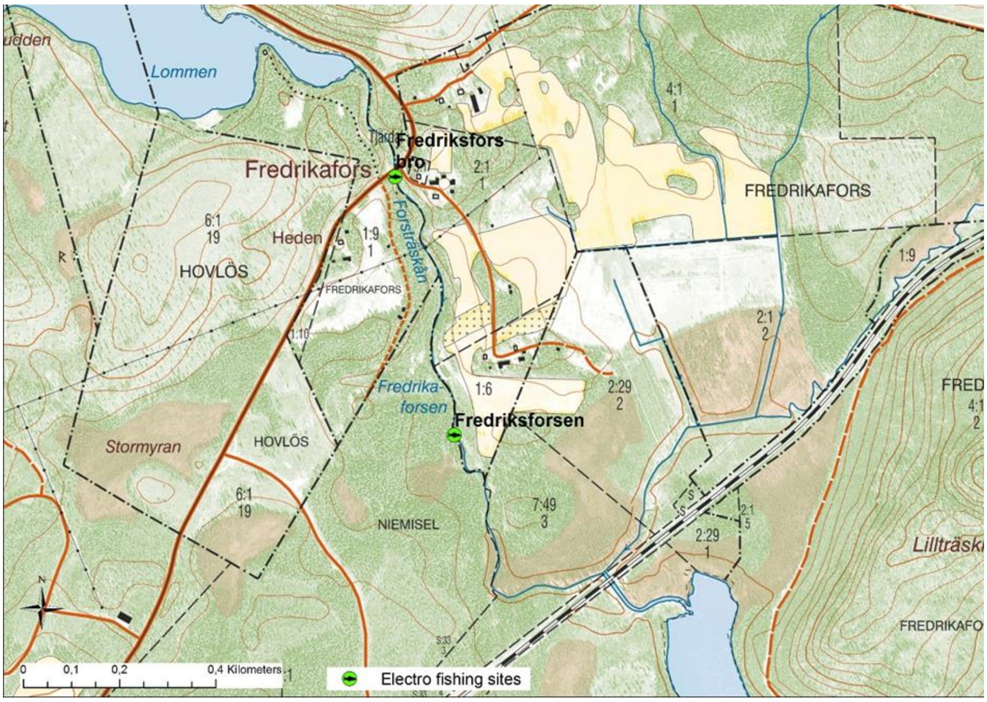

Figure 4. Location of the two different electro fishing sites in River Forsträskån. © Lantmäteriet.

The stream was investigated in 2017 at two sites (Figure 4). No trout or salmon was caught.

The FPM population consist of a few old individuals and is at risk of being extirpated (Olofsson, 2018).

The stream has not been restored.

Långträskälven

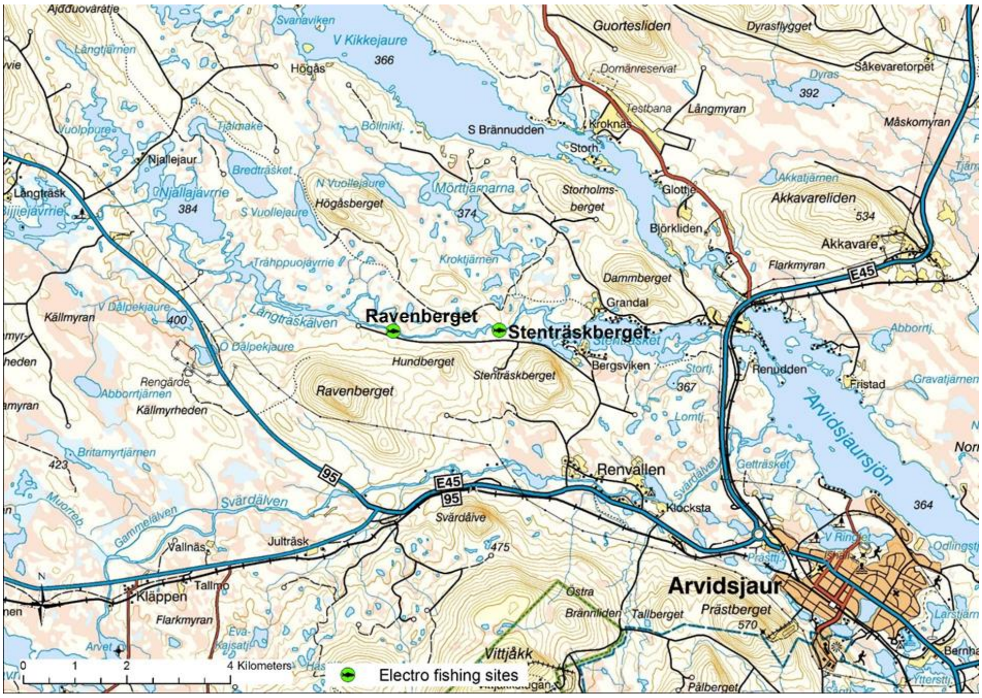

Figure 5. The location of the two sites in Långträskälven. © Lantmäteriet.

The stream has been investigated at two sites in 2017 (Figure 5). Trout was the only fish caught at both sites. No glochidia was found at any of the sites.

The population of FPM is considered extirpated (Olofsson, 2018).

Both sites have been restored during the project but no data on fish is available after the restoration.

Lögdeälven

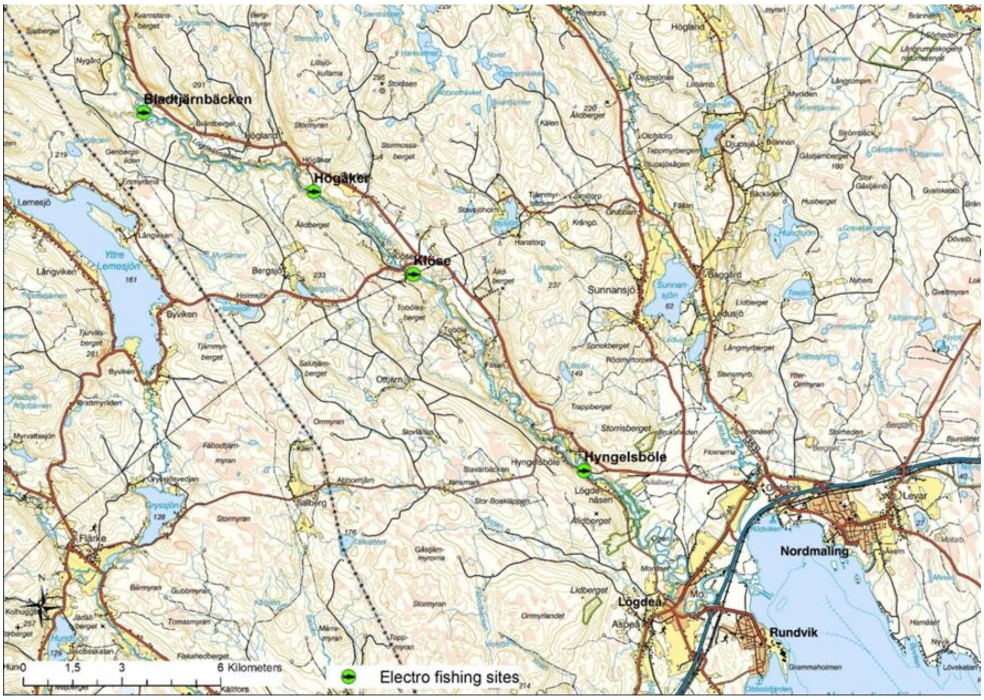

Figure 6.. Location of the three of the electro fishing sites in River Lögdeälven and the one in River Bladtjärnbäcken. © Lantmäteriet.

The stream has been investigated at seven sites in 2017, 2020 and 2021 (Figure 6, 8, and 9). Both salmon and trout have been caught and in 2020 infected salmon was found at five sites and trout at four sites. In 2021, infected salmon and trout was found at four sites. The prevalence for salmon was 42% in 2017, 48% in 2020 and 7% in 2021, and for trout the prevalence was 15% in 2017, 7% in 2020 and 12% in 2021. The decrease in prevalence between the 2020 and 2021 was significant for salmon (X^2 (1, N = 494), 102.58, p = .00001) but not for trout (p = .42). The seven different sites show a great variety in prevalence of infected salmon (Figure 7).

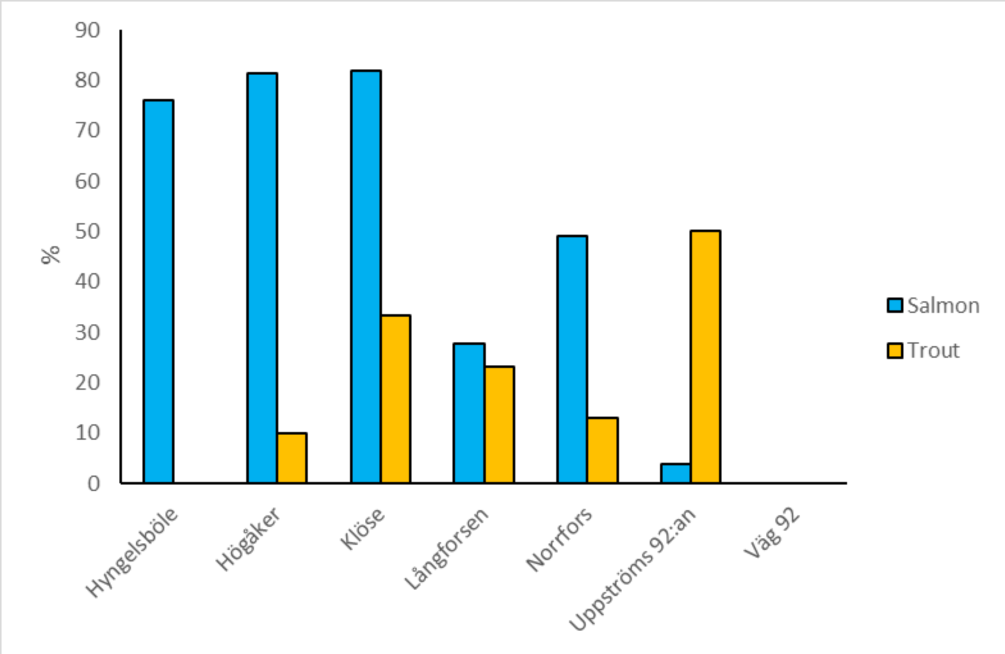

Figure 7. A comparison between the seven sites and the prevalence of infection on salmon and trout. All years are pooled.

The intensity of the infection was significantly different between the species, salmon had on average 11 glochidia per fish and trout had 1.7 glochidia per fish (Z = 5.33, $p < .00001$). The range of glochidia on salmon was 1 – 100. All trout were found with one or two glochidia each.

The FPM population do not exhibit a risk, but it needs to be further investigated. There are no signs of recent recruitment (Olofsson, 2018).

Several sites have been restored in Lögdeälven between 2015 – 2021 and three of them have comparable data from before and after the restorations (Table 3).

Table 3. Site name, restoration year, and sampling year. A weighted mean value is presented for each site and year. The pre-restorations investigations were made in May and June, three months before the restoration of the stream.

Site	Restoration year	2017	2020	2021
Högåker	2017	1.23	1.54	1.20
Långforsen	2017	0	0.5	0
Klöse	2018	1	1.67	1

Bladtjärnbäcken

The stream was investigated 2017, 2020 and 2021 at one site (Figure 6). Glochidia infected brown trout was found in 2017 and Atlantic salmon was found in 2020. In 2017 the prevalence was 10% on brown trout and the intensity was 1 glochidia per trout. In 2020 the prevalence of infected Atlantic salmon was 18% and the intensity was on average 7 glochidia per salmon. The range was between 4-10 glochidia on salmon and on trout it was one. The average length of the non-infected brown trout (*n* = 28) was 89.3 mm ± 12.6 mm, the length of the infected trout was 65 mm, salmon (*n* = 18) was 88 mm ± 13.4 mm, and the length of the infected salmon (*n* = 4) was 62 mm ± 5.5 mm.

The FPM population seems to consist of only two individuals, and they are at risk of going extirpated (Olofsson, 2018).

No habitats improvements have been performed at the site where the fish was caught.

Blåbergsjöbäcken

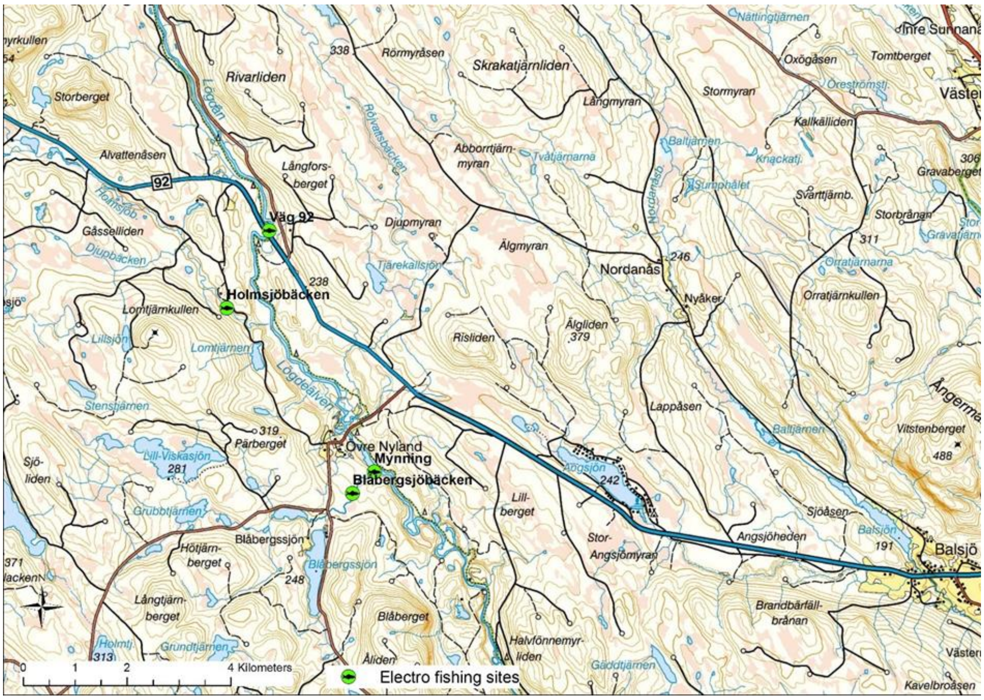

Figure 8. The sites in Blåbergsjöbäcken together with sites in Holmsjöbäcken and Lögdeälven. © Lantmäteriet.

The stream was investigated in 2017, 2020 and 2021 at two sites (Figure 8). Both Atlantic salmon and brown trout was caught but glochidia could only be found on trout. Infected brown trout was found at two sites in 2017 and the prevalence was 67%, in 2020 the prevalence of infected fish was 29%, and 69% in 2021. The intensity was on average 61 glochidia per trout in 2017, 25.5 glochidia per trout in 2020 and 98.3 glochidia per trout in 2021. In 2017 the non-infected trout was 130 mm and the length of the infected was 109 mm. In 2020 the length of non-infected trout (*n* = 4) was 141 mm ± 20.1 mm, and the length of the infected trout was 83.5 mm ± 17.5 mm, and in 2021 the infected fish (*n* = 9) was 77.7 mm ± 16.4 mm.

The FPM population was estimated to contain 6500 individuals, and there are signs of recruitment (Olofsson, 2018).

In 2018 both sites were restored but there is not enough data to analyze from 2017.

Blåtjärnbäcken

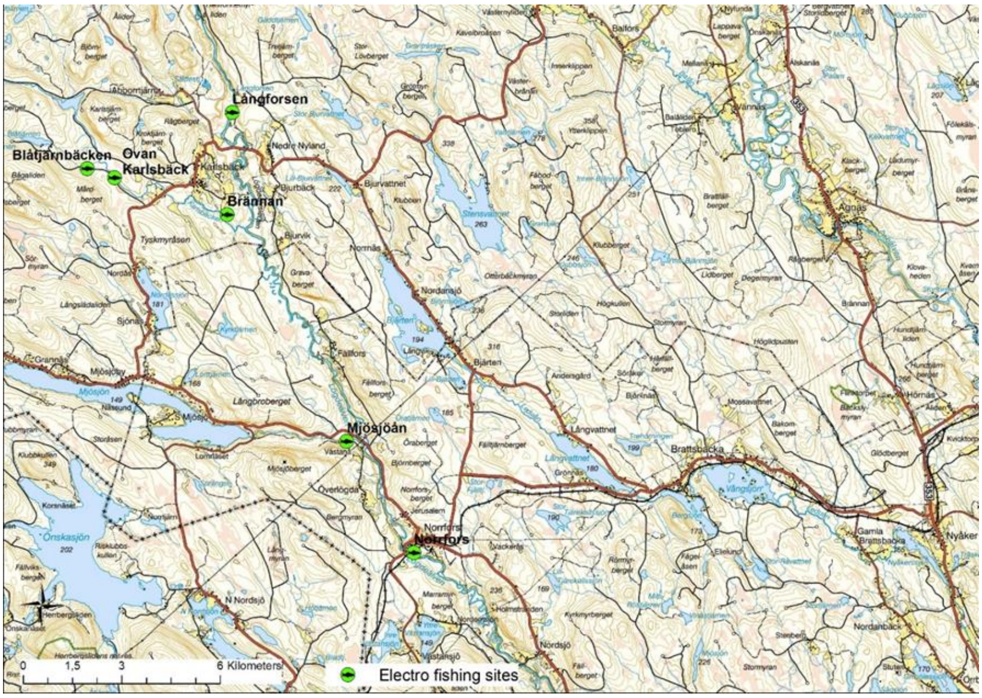

Figure 9. Location of the site in Blåtjärnbäcken together two of the electro fishing sites in River Lögdeälven, the one in River Mjösjöån and the two in River Karlsbäcken. © Lantmäteriet.

The stream was investigated 2017, 2020 and 2021 at one site (Figure 9). In 2017, trout was the only fish species that was caught, and it was not infected. In 2020, salmon (n = 6) was the only species caught and no salmon was infected by glochidia. In 2021, brown trout was the only species caught and every trout (n = 3) was infected with glochidia. Average length of the infected trout was 170.7 mm ± 77.5 mm, and the intensity of the infection was on average 67 glochidia per trout.

The FPM population is calculated to consist of approximately 1200 individuals. Juvenile recruitment occurs (Olofsson, 2018).

No habitat improvements have been performed at the site where the fish was caught.

Holmsjöbäcken

The stream was investigated in 2017 at one site, and in 2020 and 2021 at two sites (Figure 8). Each year brown trout was found to be infected with glochidia and the prevalence was 29% in 2017, 10% in 2020 and 23% in 2021, the difference between 2020 an 2021was insignificant (p = .37). The intensity of the infection was 1.2 glochidia per trout (n = 5) in 2017, 2.2 glochidia per trout (n = 12) in 2020 and 1.7 per trout (n = 3) in 2021. The number of glochidia range between 1 – 4. Average length on the infected trout was 85 mm ± 7.9 mm, and the non-infected trout was 85 mm ± 3.9 in length. The difference was insignificant (p = .79).

No FPM has been found in the stream (Olofsson, 2018), but they are there somewhere. The stream needs to be investigated.

Neither of the sites have been restored.

Karlsbäcken

The stream was investigated at two sites in 2017, and on three sites in 2020 and 2021 (Figure 9). Both salmon and trout have been caught but only trout has been infected with glochidia. In 2017 the prevalence was 32%, in 2020 the prevalence was 10% and in 2021 the prevalence was 16%. The prevalence was significantly higher in 2017 than 2020, (X^2 (1, N = 68), 5.22, p = .02), the difference in prevalence between the years 2020 and 2021 was insignificant (p = .52). Infected trout was found at two sites in 2017 but only at one site in 2020, and in 2021 trout with glochidia was found again at two sites. The intensity of the infection was low and on average the infected trout had 2.2 glochidia per trout in 2017, 1 glochidia per trout in 2020, and in 2021 the average number of glochidia per fish was 9.

No FPM have been found (Olofsson, 2018), but they are obviously there somewhere. The stream need to better investigated.

The sites have not been restored during the project.

Mjösjöån

The stream was investigated at one site in 2020 and 2021 (Figure 9). In 2020 both salmon and trout were caught but neither of them was infected. In 2021 one salmon were caught but it was not infected.
No FPM has been found in the stream (Olofsson, 2018).

The stream was restored during the project, but no fish has been caught carrying glochidia.

Rutnajoki

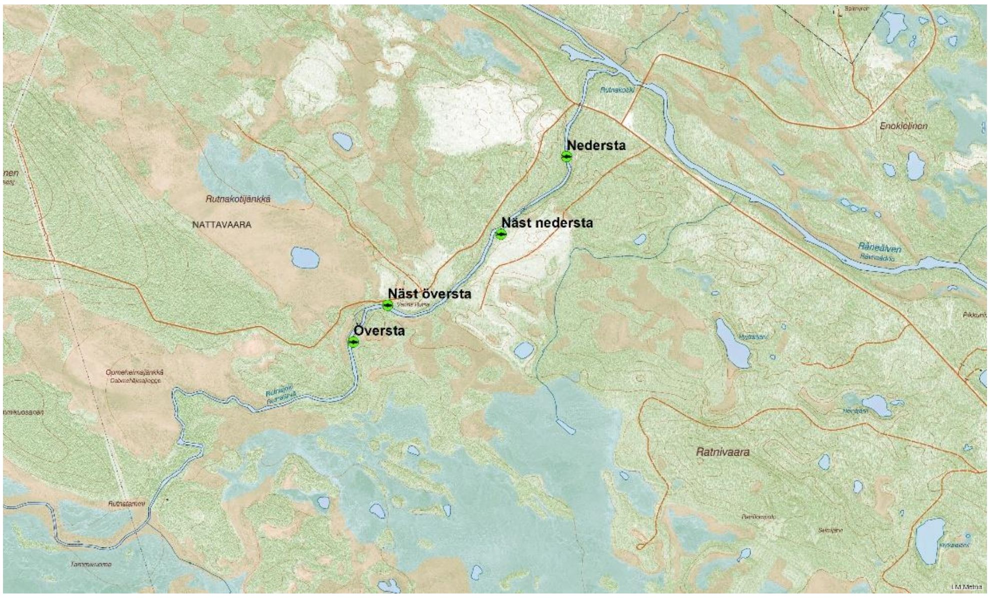

Figure 10. Location of the four different electro fishing sites in River Rutnajoki. © Lantmäteriet

The stream was investigated at four sites in 2020 and 2021 (Figure 10). In 2020, two trout were caught neither of them were infected. In 2021, two trout were caught, one of them were infected with 75 glochidia.

In Rutnajoki the FPM population is estimated to consist of >400 individuals. A few juvenile mussels have been found during the project. The population is at risk of being extirpated.

The stream was restored during the project. The lack of data makes it impossible to make any analysis.

Råneälven

Figure 11. Location of the four different electro fishing sites in River Råneälven. © Lantmäteriet.

The stream was investigated at four sites in 2017, 2020, and 2021 (Figure 11). Only salmon was caught during the years and the prevalence was 81% (n = 43) in 2017, 92% (n = 48) in 2020, and 91% (n = 21) in 2021. The difference in prevalence between all sites was not significant (p = .09) between 2017 and 2020, and the same between 2020 and 2021 (p = .88). The average intensity of the infection was 10 glochidia per salmon in 2017, 14.1 glochidia per salmon in 2020, and 9.7 glochidia per salmon in 2021. There was no significant difference in length between the non-infected (N = 16, 75.8mm ± 11.7 mm) and infected (N = 99, 67.1mm ± 3.5mm) salmons.

The size of the population of FPM in Råneälven is unknown but juvenile FPM has been found on different locations in the stream (Olofsson, 2018).

The stream has been restored in 2019 at several sites and one of the sites (Snasko, nedre) has been investigated for prevalence and intensity of the infection. In comparison, with the three other sites (controls) the prevalence is like the controls (Figure 12).

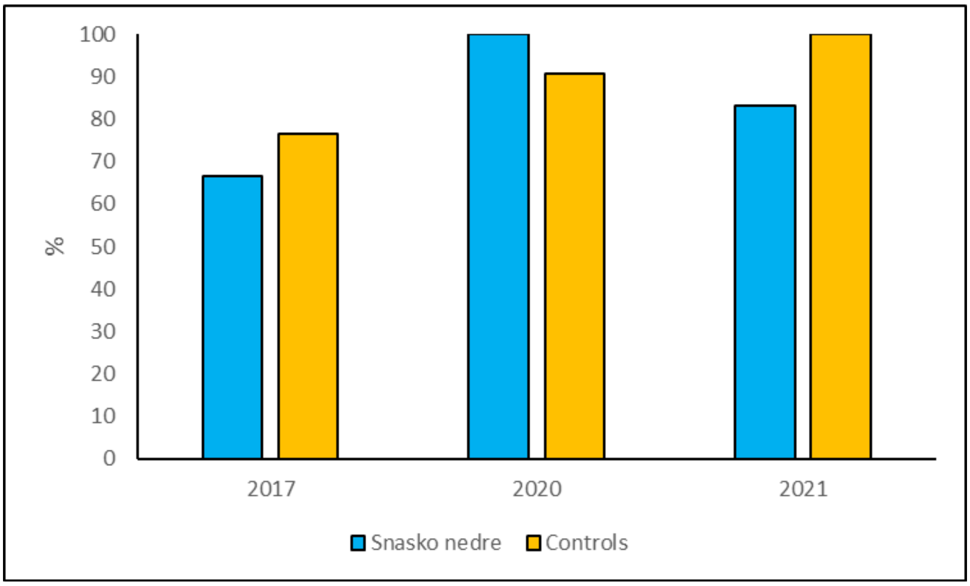

Figure 12. A comparison of prevalence between the restored site Snasko nedre and the controls between the years 2017, 2020, and 2021.

The average intensity of infection seems to have increased between the years 2017, 2020, and 2021 at the site Snasko nedre, but the difference is not significant (*p* = .65) (Figure 13). At the controls the difference in average intensity was significant between the years (F (2, 74) = [23.3], *p* <.0001).

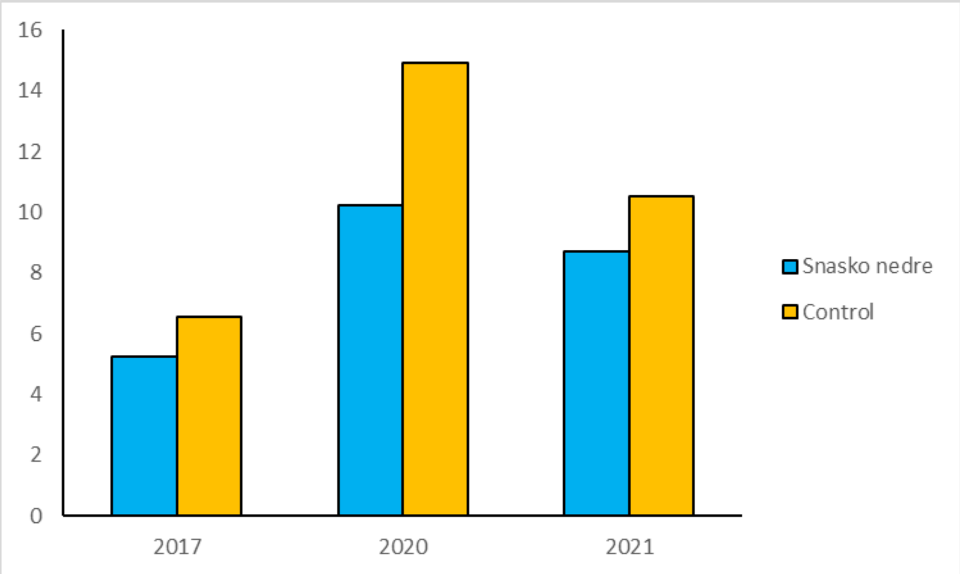

Figure 13. A comparison of the infection intensity bewteen the years 2017, 2020, and 2021, at the site Snasko nedre and the controls.

Vitbäcken

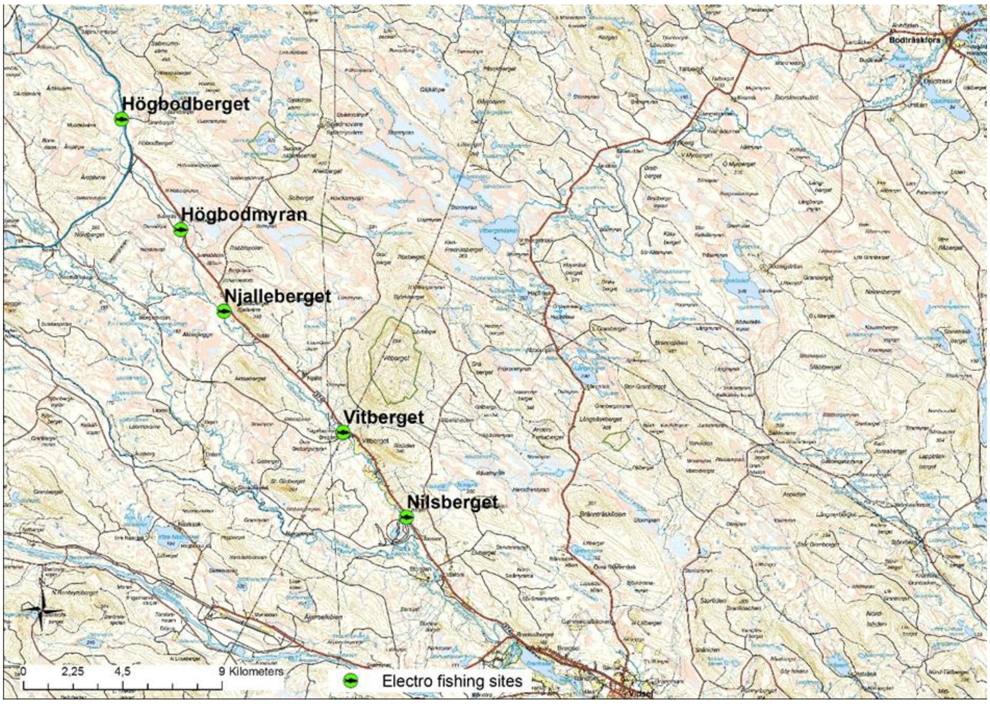

Figure 14. The location of the five sites in Vitbäcken. © Lantmäteriet.

The stream has been investigated at 5 sites between the years 2017, 2020, and 2021(Figure 14). Both salmon and trout were caught but only trout was infected. In 2017 infected trout was found at three of the sites, and in 2020 and 2021 at only one site. The prevalence was 27% in 2017, 25% in 2020, and 33% in 2021, for all sites. The difference between 2017 and 2020 was insignificant (p = .9) and the same for 2020 and 2021 (p = .8). The average infection intensity was 7.3 glochidia per trout in 2017, 1 glochidia per trout in 2020, and 2 glochidia per trout in 2021.

The population of FPM in Vitbäcken is at risk of being extirpated, but there is sign of recent recruitment (Olofsson, 2018).

The stream has been restored at four of the investigated sites, unfortunately the lack of data makes it impossible to do any analysis.

Åbyälven

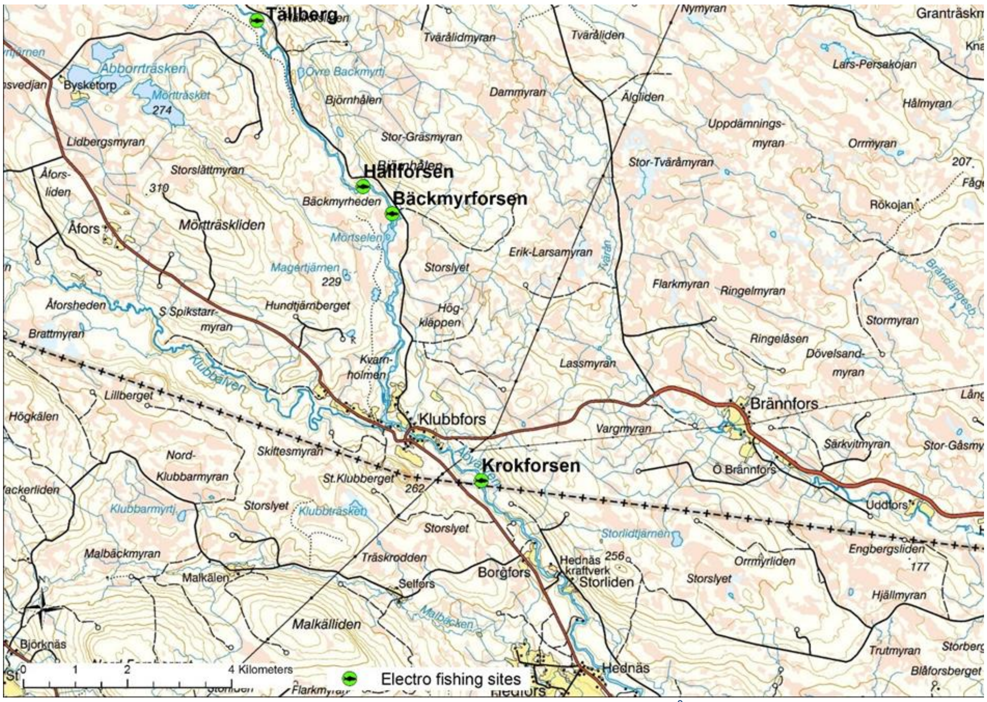

Figure 15. Location of the four different electro fishing sites in River Åbyälven. © Lantmäteriet.

The stream was investigated at four sites between the years 2017, 2020, and 2021 (Figure 15). Both salmon (N = 35) and trout (N = 1) was caught but only salmon were infected. Infected salmon was found at one site in 2017 and on two sites in 2021, in 2020 no salmon was found to be infected. Prevalence for salmon at all sites was 25% in 2017, and 46% in 2021, the difference was insignificant (p =.23). Prevalence for trout was 100% in 2021. The average infection intensity was 1.3 glochidia per salmon in 2017, and 1.2 glochidia per salmon in 2021. The trout was infected with 2 glochidia.

The FPM population in Åbyälven consist of a few elderly individuals and there is no sign of recent recruitment. The population is at risk of being extirpated (Olofsson, 2018).

The stream has been restored at multiple sites but it is impossible to detect any significant differences in prevalence or intensity.

Discussion

The results from the investigation cannot answer the hypothesis that stream restoration would increase the prevalence and intensity. This is due to the lack of data and probably time, both in sample sizes and time before and after the restorations. However, the data collected, no matter how small the numbers are, can be valuable baseline data to future studies. Perhaps prevalence and infection intensity need to be investigated during a longer period before any obvious patterns are revealed?

The hypothesis that both prevalence and infection intensity should increase when a stream has been restored is based on the theory that the in-stream residence time for water should increase as boulders, rocks, and dead wood are added to the stream channel, this would also make the in-stream residence time increase for the glochidia, which in turn would enhance the chance to get more fish host infected (Worrall et al., 2014). However, prevalence and infection intensity are factors that also are positively influenced by the abundance of FPM (Österling et al., 2008). The influence of mussel abundance on prevalence and infection intensity is probably stronger than the increase of in-stream residence time, but this hypothesis needs to be further investigated. The abundance of FPM in the streams is not large, and that can be one reason why the prevalence and the infection intensity was low. However, in two of the streams the knowledge about FPM was insufficient as it turns out that fish was infected. This proves that electrofishing for infected fish is a tool to detect new populations (Salonen & Taskinen, 2017). One goal with this project was to enhance the abundance of FPM, so, with longer time series of investigations all these streams can work as valuable experimental sites to draw conclusions from in the future.

The investigations have revealed that some of the streams in the project inhabited by FPM utilize Atlantic salmon as the primary fish host. Together with the first report (Olofsson, 2018) and the data presented in this report we have the first records of salmon as fish host to the FPM in Sweden. Even though the infection intensity was very low in some of the streams, the fact that all data was collected in the early summer and close to the time of excystment, makes these investigations reliable in terms of fish host determination in each stream (Österling & Wengström, 2015). Interestingly a few streams also had infected trout at the same site as infected salmon was caught. However, the infection intensity on the species was different with very low numbers of glochidia on the trout compared with the numbers on salmon. The phenomenon that both salmon and trout are infected at the same site has to my knowledge only been reported before from a few streams in Scotland (Hastie & Young, 2001). There are investigations from Norway where salmon and trout co-exist together with FPM but were only one of the fish species have been found with glochidia infection (Karlsson et al., 2014). According to the Norwegian article there should be a genetic difference between FPM that use salmon as host and FPM that use trout as host, this may be a useful tool to use in streams where no infected fish was caught (Karlsson et al., 2014).

This was the last year that these kinds of investigations would be performed in LIFE ReBorN. I would like to give some recommendations to future investigations on prevalence and infection intensity on fish hosts.
The timing of the investigation – The preferable time of the year to do these investigations are as close as possible to the time when the glochidia fall off the host. At that time the glochidia are at their maximum size which makes them easy to see and count. However, it is possible to do the investigations in the fall, Hastie & Young (2001) did their study between September – November. If the water is inhabited with other mussel species, e.g., *Unio sp* or *Anodonta sp*, it is wise to do this investigation in the fall, when no other species of glochidia than FPM can be found on a fish host. *Unio sp* and *Anodonta sp* species release their glochidia between spring and early summer, and it can be difficult to distinguished them apart from FPM glochidia if the observer is less experienced.
Number of fish collected – The number of fish you need to do a statistical test depends on the test but as a rule of thumb, try to get at least 15 fish from each site.

It is difficult to implement investigations in the field when conditions are not favorable at all time. In these investigations personnel had to struggle with high floods and days without any catch of infected fish host. These obstacles are hard to get by when resources are limited. Time is probably one of the most important components to consider when evaluating conservation actions and biological effects from such, as biological systems can have a very slow pace of life.

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