Handbook of guidelines for River lamprey monitoring in Lithuania and Kurzeme Region, Latvia

Project: Cross-boundary evaluation and management of lamprey stocks in Lithuania and Latvia
LAMPREY LLI-310

Institute of Food Safety, Animal Health and Environment "BIOR"
Rīga, Latvija

2020

Document is produced by Institute of Food Safety, Animal Health and Environment "BIOR" and Klaipeda University within INTERREG project LAMPREY Cross-boundary evaluation and management of lamprey stocks in Lithuania and Latvia LLI-310.

AUTHORS for development of guidelines and handbook:
Kaspars Abersons, Jānis Bajinskis, Robertas Staponkus, Andris Avotiņš and Elīna Ellere

Other contributions (field works, office help, useful comments and other)
Santa Purviņa, Nerijus Nika, Edmunds Bērzinš, Ruta Medne, Andrius Skersonas, Amanda Lazdiņa

Cover photo:
Santa Purviņa, the monitoring of lamprey larvae in 2019 in River Venta.

The Interreg V-A Latvia – Lithuania Cross Border Cooperation Programme 2014-2020 aims to contribute to the sustainable and cohesive socio-economic development of the regions of the Programme by helping to make them competitive and attractive for living, working and visiting.
This project is funded by the European Union. The total project's size is EUR 353 400. Out of which the co-funding of the European Regional Development Fund is EUR 300 390. Project duration: 01.04.2018. – 31.09.2020.
This document has been produced with the financial assistance of the European Union. The contents of this document are the sole responsibility of Project and Institute BIOR and can under no circumstances be regarded as reflecting the position of the European Union.

Limitation of Liability and Rights

Table of Contents

Introduction

River lamprey *Lampetra fluviatilis* is one of the most important species in Latvian inland waters fishery and considerable amount of river lamprey has been caught in Lithuania as well. Results of socio-economic studies performed within the LAMPREY project confirms that the river lamprey is an important source of income for fisherman and is also valuable for the protection of traditional lifestyle in Kurzeme Region. In addition to commercial and cultural value the river lamprey is also important for nature protection. The species is listed in both Annex II and Annex V of Council Directive 92/43/EEC on the conservation of natural habitats and of wild flora and fauna as well as in several other national and international legislative acts of nature protection. Exploitation of river lamprey in the EU is allowed only if member states ensure that the population of this species is maintained at a favourable conservation status and therefore a relevant monitoring of status of this species is needed. Availability of reliable information of actual status of river lamprey population is also a keystone for planning and implementation of relevant management and maintenance measures for this species. In the same time the effort needed for collection and analysis of monitoring data should be as small as possible and consume as little resources as possible.

River lamprey has a complicated life cycle which includes the larval stage in freshwater, true metamorphosis, migration to the sea, feeding in the sea, spawning migration to freshwater and spawning itself. The methods for monitoring of each part of life cycle is different (Moser, 2007). This document provides guidelines for monitoring of the lamprey larvae and monitoring of the number of adults migrating upstream which at the same time will also allow the estimation of fishing mortality rate of the spawning stock. These two methods cover most part of the river lamprey life cycle thus providing the relevant information on status and exploitation of this species. At the same time both methods are relatively simple and cost efficient. In addition to that, this document also contains the guidelines for evaluation of the success of lamprey maintenance measures. The information on the success rate is crucial for continued increase of the efficiency of such measures.

Guidelines included in this document are based on the deliverables of the LAMPREY project (Deliverable T1.5.1. Output OT1.2 “Protocol for monitoring of lamprey larvae”; Deliverable T1.5.1. “Protocol for monitoring of the number of lamprey spawners entering rivers (mark-recapture survey)” and Deliverable T1.3.1. “Methods for monitoring the stocking efficiency of lamprey larvae or translocation of lamprey spawners”). Elaboration of these deliverables is based on findings of LAMPREY projects as well as on the results of previous lamprey research in Latvia and Lithuania and relevant literature.

We encourage to use these guidelines not only for monitoring within the Lamprey project region, but also in other parts of Latvia and Lithuania as well as in other countries.

1. Guidelines for monitoring of lamprey larvae

1.1 Background information and rationale

Existing lamprey larvae monitoring methods can be divided in two large groups where one group includes methods where lamprey larvae are captured by electrofishing while other group consists of methods where capture of lamprey larvae is done by bottom sampling. So far lamprey larvae in Latvia and Lithuania have been collected by bottom sampling and in order to ensure the compatibility with the existing data, the bottom sampling should be continued. However, the sampling gear used for the capture of lamprey larvae until the 2018 was different in each country. In Lithuania bottom samples were taken by sampling gear first described by Lasne *et al.* in 2010. This gear is the 55 cm high and 30 x 40 cm wide box with an open top and bottom and nylon netting with 0.5 mm mesh size attached at one side (Figure 1.1.). At the same time in Latvia the bottom samples were taken by specially designed 8 cm high, 25 cm long and 20 cm wide shovel (Figure 1.2). Both methods have their own advantages and drawbacks. Greatest advantage of the sampling box is that the precise area of each sample is known. Yet due to its relatively large size the operation of this box is limited in reaches dominated by stone or wood or another large substratum. Shovel is smaller and more adjustable, yet it is very hard or even impossible to measure the exact length for each sample or transect and estimate the size of sampled area.

Figure 1.1. Box for sampling of river lamprey larvae described by Lasne *et al.* 2010 and used for lamprey larvae monitoring in Lithuania

Figure 1.2. Shovel for sampling of lamprey larvae which until the 2018 was the primary tool for lamprey larvae monitoring in Latvia

One of activities of LAMPREY project was the intercalibration of both methods. Results of intercalibration confirmed that both methods produce compatible results (Figure 1.3) yet the Lithuanian or box method demonstrates the better value distribution and theoretical concept for the monitoring data analysis. Therefore, it was concluded that further on the bottom sampling box described by Lasne *et al*. 2010 must be considered as primary method for monitoring of lamprey larvae in Latvia and Lithuania.

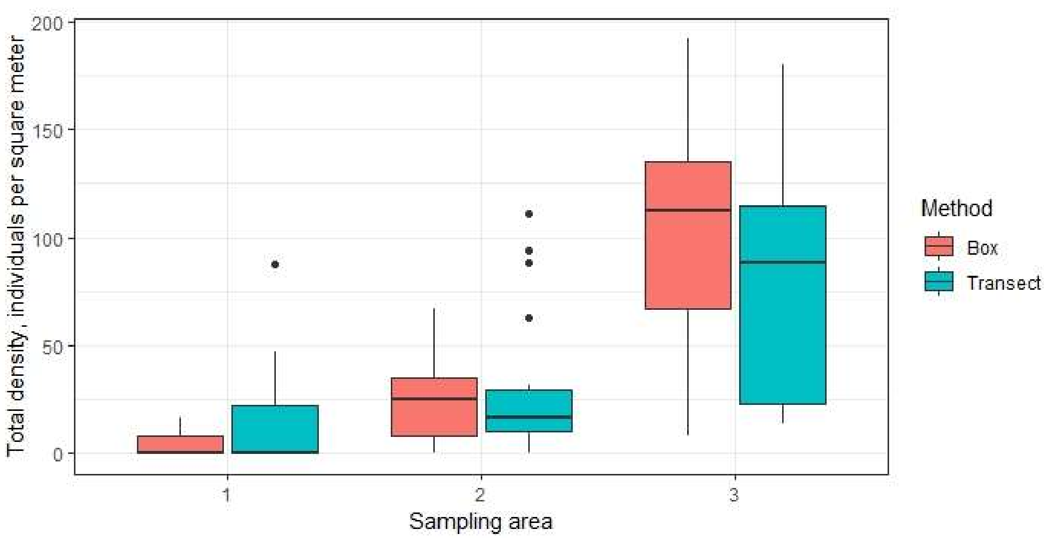

Figure 1.3. Comparison of the results of lamprey larvae density in three sampling sites obtained with Latvian (shovel or transect) and Lithuanian (box) methods

In accordance with the project application, the method used for further lamprey larvae monitoring should be sensitive enough to detect population change by factor 1.5 with 80% power at 95% significance. As it was concluded during the intercalibration of Latvian and Lithuanian sampling methods such accuracy can be reached if in the territory of interest there are at least 12 samples taken in at least 40 sampling sites. Since the project area includes the territory of both Latvia and Lithuania, at least 40 sites must be sampled in each of these countries. This will give the opportunity to follow the changes in each country. In order to obtain monitoring results that accurately resemble changes in the lamprey population, sampling sites must remain fixed, and monitoring must be conducted at same sites every year. Sampling sites must be located in all greatest rivers of the region, and in their tributaries in reaches that are suitable for lamprey larvae.

Abundance of lamprey larvae varies in different types of habitat. To increase the quality of gathered data samples in each sampling site must be positioned so that they cover both optimal and suboptimal biotopes for lamprey larvae. If possible, the number of samples in each of habitat type must be proportional to the amount of such biotopes in the sampling site. Analysis of the existing Latvian lamprey monitoring data indicates that river biotopes can be grouped in 4 main categories (Figure 1.4).

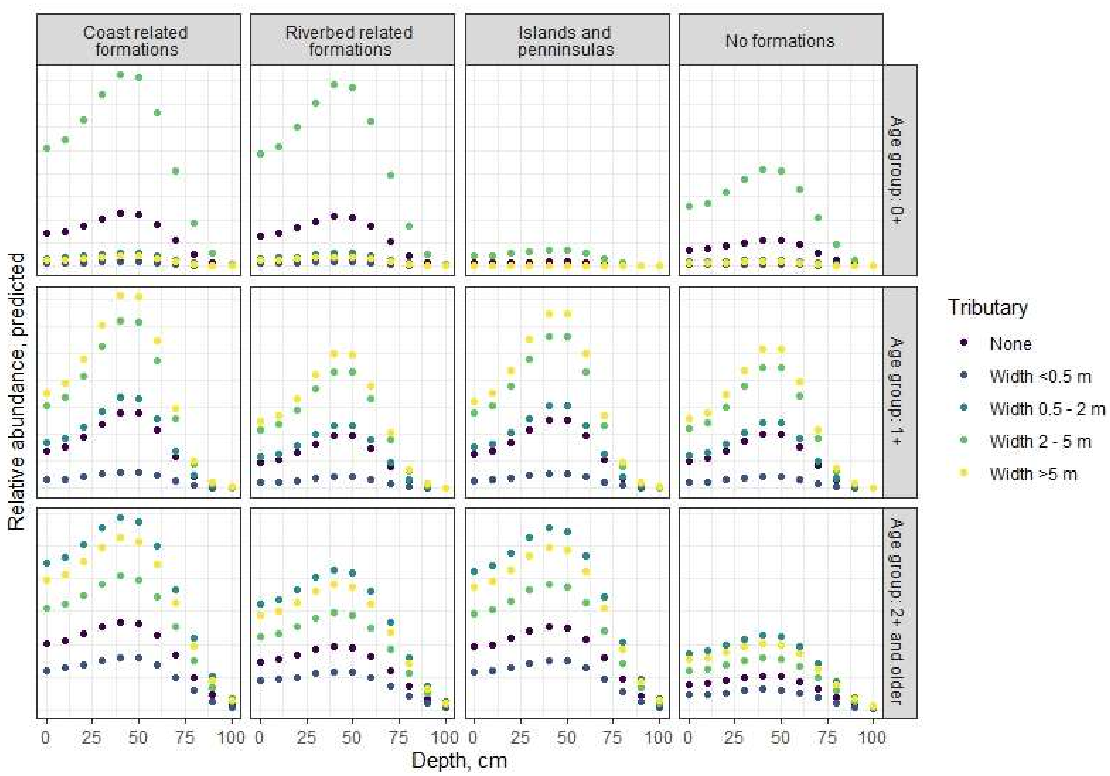

Figure 1.4. Predicted abundance of lamprey larvae in different categories of habitats

These four habitat types are: coast-related formations (i.e. coastal slope of riverbed, bends and curvatures of riverbank, and other formations in which riverbed is directly related to coastline); riverbed-related formations (i.e. slopes of pits of riverbed, sediment deposits before and after rocks, trees or other obstacles etc.); big formations (i.e. riverbanks and sediment deposits associated with islands or peninsulas) and straight riverbed with no obvious formations and not related to coastline. The abundance of lamprey larvae of different age groups in each of these categories of formations is slightly different. Occurrence of larvae in sampling site is also affected by river depth, presence of tributary of different size, age of larvae and other factors. Considering the different occurrence of river lamprey larvae in previously mentioned formations, it is important to ensure that the location of samples in each sampling site is proportional to the share of these formations in particular site. To increase the value of gathered data the most important parameters must be registered during the sampling. On the basis of data from the annual Latvian lamprey monitoring 2014–2018, a form for registration of the relevant information was elaborated (see the appendix) and successfully tested during annual monitoring of lamprey larvae in 2019 and 2020.

Our experience shows that it is relatively easy to identify first two age groups on the basis of length data, however borders for other length groups are disputable (Figure 1.5). Therefore, we suggest dividing larvae in only three age groups: subyearlings (0+), yearlings (1+) and older larvae.

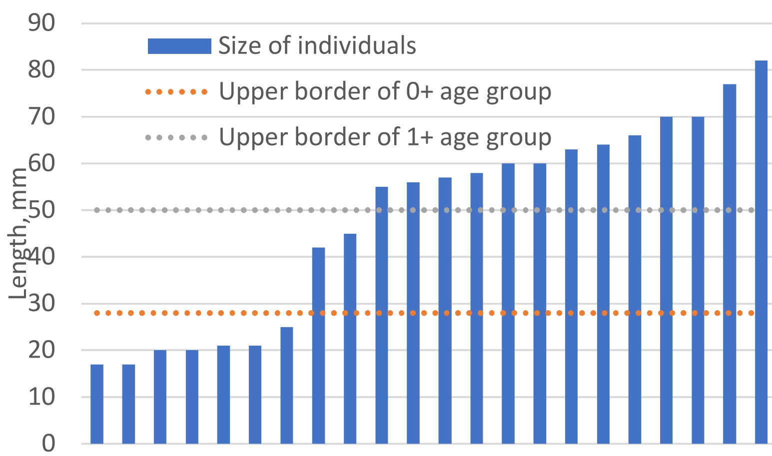

Figure 1.5. Size of individual lamprey larvae and suggested border of age groups in sampling site located in River Pēterupe

Growing speed of lamprey larvae in each river is different. So, in coldwater streams where the water temperature rarely exceeds 16°C the upper border for size 0+ larvae is approximately 20 mm and for 1+ age group – 45 mm. Meanwhile in the warmer streams the upper border can reach 30 mm for 0+ and 60 mm for 1+. Growth speed can also be influenced by availability of nutrients and other factors therefore the categorization into age groups must be performed for each river (preferably for each sampling site) separately. To detect all age groups sampling must be done in August-September when 0+ larvae is big enough to be successfully detected.

1.2 Guidelines for monitoring of lamprey larvae

1.2.1 Method and equipment

Recommended method for monitoring of lamprey larvae in Latvia and Lithuania is the bottom sampling with the sampling box Lasne *et al.* 2010.

1.2.2 Sampling sites, samples and timing for sampling

40 sampling sites should be sampled in area of interest and 12 samples should be taken in each sampling site. In territory of LAMPREY project there are two areas of interest – Lithuania and Kurzeme Region in Latvia – and 40 sites should be sampled in each of these areas. Sampling should be performed in the same sites each year and, if possible, also on the same time as the previous years. Sampling sites should be situated in river reaches suitable for lamprey larvae and freely accessible for anadromous migration. It is recommended to avoid the establishment of the sampling sites in rapids or other reaches dominated by rocks, pebbles, clay or other substrate that prevents burrowing of larva as well as in reaches with low dissolved oxygen concentration and other places clearly unsuitable for lamprey larvae. Recommended rivers to be sampled and number of sampling sites in each river for Kurzeme Region is compiled in Table 1.1. Sampling should be done in August or September.

Table 1.1. Recommended rivers and number of sampling sites for lamprey larvae monitoring in Kurzeme

In Kurzeme Region, Latvia					
No.	Name of river	No. of sites	No.	Name of river	No. of sites
1	Grīva	1	14	Abava	2
2	Roja	3	15	Padure	1
3	Pilsupe	2	16	Riežupe	2
4	Melnsilupe	1	17	Užava	1
5	Pitragsupe	2	18	Kauliņa	1
6	Mazirbe	1	19	Vanka	1
7	Irbe	2	20	Rīva	1
8	Lonaste	1	21	Tebra	2
9	Raķupe	1	22	Durbe	2
10	Pāce	1	23	Akmene	1
11	Venta	5	24	Vārtāja	1
12	Vēždūka	2	25	Virga	1
13	Dzirnavupe	1	26	Ruņa	1

Samples in each sampling site should be located in such way that they are proportional to the share of the main habitat groups (i.e., coast-related formations, riverbed-related formations, formations related to islands and peninsulas and straight riverbed with no formations, for more information see Chapter 1.1). For example, if approximately 50% of the sampling site consist of coast-related formations, 25% of the site are riverbed-related formations and another 25% are riverbeds with no formations, then 6 samples should be collected from coast-related formations, 3 from riverbed-related formations and 3 from riverbed without formations. If possible, samples in each type of formation should be collected at 3 depth zones: up to 35 cm, a zone of 35 – 55 cm depth, and greater than 55 cm. Number of samples in first two depth zones must be proportional to the share of these depth zones in sampling site. Deeper zone needs to be sampled only if there are considerable share of habitats suitable for lamprey larvae.

1.2.3. Process of sampling

Immediately after arrival at the sampling site, the first page of the date registration form (recommended registration form can be found in appendix of this document) dedicated to the description of the sampling site is filled in. The upper part of the form includes the overall information – number of the sampling site, date of sampling, name of the river, the respective river catchment, geographic coordinates and description of location (e.g. downstream bridge of the road V387).

Below the general information is the overall description of the sampling site (part of the river where the sampling will be performed) and the adjacent part of the river (as far as it can be seen from the sampling site). The first table includes fixed proportion (sum=100%) of respective depth zones (<35; 35-55; >55 cm) in the sampling site and the adjacent part of the river. The approximate proportion of depth zones is decided by expert judgement. The average width of the stream is measured accurately if possible or estimated approximately if accurate measurement is not possible (usually depends on the size of the river). In smaller rivers (maximal depth is less than 1 m, and width is no more than 6 m) sampling should be performed across the entire riverbed and therefore, the width of the sampling site equals the width of the river. The coverage of macrophytes (algae, nympheids, elodeids, helophytes and lemnids) is estimated as a percentage (sum≠100%) and recorded in the form. Information on respective groups of macrophytes are specified below the table.

Next step includes recording the category of stream velocity and presence of tributary in the sampling spot or just upstream of it. Categories of stream velocity are as follows: “0” – there is no visible water movement in the sampling plot or within sight; “1” – water flows in the sampling plot or within sight, but does not exceed 0.2 m/s; “2” – within sight there is a section, where stream velocity exceeds 0.2 m/s; “3” – the stream velocity exceeds 0.2 m/s before and after sampling plot, but within sight there are also slower sections; “4” – the stream velocity exceeds 0.2 m/s everywhere or almost everywhere.

Width of tributaries should be registered in the form and if there are no tributaries, the respective box should be marked with "–". Anthropogenic impact at the sampling site and the adjacent part of the river should also be indicated in the form. Three types of anthropogenic impact must be registered: recreational and other activities (wading, boating or swimming), digging of a stream channel (e.g. for melioration or other purposes), or an object (e.g. bridge, embankment). The proportion (%) of the sampling site and the adjacent part of the river affected by the particular type of impact must be indicated. The distance to the dam or other migration obstacle must also be recorded. After sampling, the available databases must be searched to ensure that there are no dams or hydroelectric power stations located outside the visibility range from the sampling site.

Next, the dominating substratum in the stream should be estimated. It might be unsuitable for lampreys (e.g. clay, dolomite), stony (share of stones should exceed 50% and average diameter of stones must be at least 20 cm), mixed (stone from 30% to 50%, sand and gravel), sand and gravel (share of gravel is at least 5%), or soft (dominated by sand and/or detritus, the share of gravel <5% and stones <30%). Only one category can dominate, therefore only one is marked in the protocol. If the sampling plot is the same size as the river section, it is not necessary to mark both boxes.

Finally, the pH level, conductivity (µS/cm), dissolved oxygen (mg/l) and water temperature (°C) is assessed and registered in the form. In each site, a photography of river section is taken, and the time of this action recorded in the form.

After that, the sampling takes place and the data regarding samples and captured lamprey larvae is registered. Second page of the data registration form contains a large table, that combines the characteristics of each sample. A total of 12 bottom samples should be taken. Each sample is already numbered, but all other sections must be marked during the sampling. As mentioned previously, location of samples must be proportional to riverbed heterogeneity groups and to depth zones within each of these groups within the sampling site. Location of each sample should be considered before the beginning of sampling, and sampling should be started with the closest sample. **NB! Do not sample in places you have previously disturbed by wading, standing, washing of samples or in any other way.** This must be considered when deciding on the position and sampling order for the individual samples.

Sampling procedure itself is described by Lasne *et al.* 2010. In general, sampling procedure consists of pushing the box into the riverbed and digging sediment into net bag fixed to one side of the box. Then substrate is washed through the same net and larvae remaining in net removed. To ensure that there are no larvae left in the box, it should be dip-netted before and after removal of sediments. For each sample the average depth is measured and recorded in the respective box (<35 cm; 35-55 cm; 55< cm) as well as the category of slope of the riverbed (from “0” – horizontal or slightly sloping, to “3” – vertical or almost vertical slope) and coverage of different groups of macrophytes (sum can differ from 100%). If the sample location overlays with some anthropogenic activity/impact (e.g. sample is located exactly where boats have been dragged out of the water) it must be marked in the table. Approximate share of different type of substrate (organic, sand, gravel and stone) should also be evaluated. We propose for the person performing the sampling to approximately estimate the amount of substratum in the sampling net before and after washing and to evaluate the proportion of sand, that has been washed from the sample, after taking the bottom sample. Then approximate proportion of organic substrate, sand, gravel and stone remained in the net should be estimated. For example – bottom sample is estimated to weigh approximately 5 kg. After the washing, the leftover sample weighs about 2 kg, meaning 3 kg or 60% of sand disappeared from the sample. After examining the contents of the sample, it appears, that there are some remaining stones and gravel compiles most of the sample and organic matter is slightly less than gravel. In the table sampling person writes organic matter – 13%, sand – 60%, gravel – 17% and stone – 10% (sum=100%).

Captured lamprey larvae should be sedated (we successfully used solution of clove oil) and measured to nearest millimetre. After measuring, larvae should be placed in clear water to recover and after recovery released back in the river. **NB! If larvae are released before sampling is finished, release them at least 10 m (preferably – more) downstream from the actual sampling site**. Length data for each larva is recorded in the table of ammocoetes, which is the third section of the data registration form. First column of this section contains the number of samples in which each larva has been found. The second column is for individual number of particular larvae in this sample. The length of larva (mm) is registered in the third column, and the last column is left for notes (e.g. the larva has started metamorphosis or other).

After all of the samples have been collected, the sampling site should be characterized using transect method. The number and length of transects depend on the size of a particular sampling plot. The first transect is located at the beginning of the sampling plot, perpendicularly to the coastline. First observation of the transect is done 1 m from the coastline. There the stream velocity (m/s) and depth of the water (cm) are measured. The person performing the measuring evaluates the proportion of stones, pebbles, gravel and sand in the radius of 1 m (sum=100%) and the coverage of coarse and fine organic matter, which can overlay (sum≠100%). Braun-Blanquet scaling is used to assess the coverage of macrophytes, such as algae, helophytes, nympheids, elodeids and lemnids. The scales are: “+”, which means less than 1% of this plant group is present at the site; “1” with 1 – 5 % present at the site; “2” with 5 – 25% present; “3” with

25 – 50% present; “4” with 50 – 75% present and “5” with 75 – 100% present at the site. All of these plant coverages can overlay (sum≠5).

1.2.4. Data analysis

Only length data for individual larvae will be available after sampling. On the basis of length data all captured larvae should be divided into three age groups – subyearlings (0+), yearlings (1+) and older larvae. Growing speed in different sites is variable therefore we strongly suggest that categorization into age groups is performed for each river (preferably each sampling site) separately. Nonetheless in most occasions the upper boarder for 0+ age group can be found between 20 mm and 30 mm and for 1+ age group between 45 mm and 60 mm.

Population dynamics can be analysed and trends classified with well-established and simple methods for monitoring data analysis, i.e. TRIM (Pannekoek and Strien, 2005) and MSI (Soldaat et al., 2017). This analysis should be done for each age class separately.

In the future, once a larger amount of data is gathered, a method for calculation of environmental capacity of lamprey larvae should be developed. Such method in addition to monitoring of changes of population will also allow to estimate the overall status of the population.

.

2. Monitoring the number of upstream migrating adults and fishing mortality rate during spawning migration

2.1 Background information and rationale

River lamprey is anadromous species that is fished only during the spawning migration, therefore the fluctuation of annual landings at some extent illustrates the changes in population as well (Eglīte, 1961). In both Latvia and Lithuania lamprey fishermen are obligated to report the size of the catch after each fishing activity thus providing relevant agencies with highly detailed fisheries data. However, the size of the catch is determined not only by the status of the lamprey population. An important role is also played by meteorological and other factors which affect the intensity of spawning run as well as the fishing opportunities this way influencing also the size of annual landing (Abersons, Birzaks, 2014). Value and usability of catch data can be greatly increased if the actual proportion of migrating lampreys that are caught by fishermen is estimated. A most suitable method for evaluation of actual fishing pressure is a mark-recapture study.

The precision of results increases significantly when the size of the sample is increased. At the same time the increase of sample size increases the effort needed for monitoring, compromises the welfare of lampreys and to some extent complicates the tagging itself. Tagging survey performed within LAMPREY project and previous surveys in the Gulf of Riga suggests that current efficiency of river lamprey fishing gears is approximately 40%. Since the gear limit in Latvia has been relatively stable during the last decades it can be concluded that such exploitation rate is safe for the status of population and should not be exceeded. Therefore, the sample size should be great enough to allow detecting the 10% difference from 40% with 95% confidence level and 80% test power.

Calculations of the sample size were carried out for proportion test following the methodology by Dalgaard (2002) with software for statistical computing R (R Core Team, 2019). The larger the difference between observed and specified proportions, the smaller the sample size needed for obtaining statistically significant differences from 40%. Therefore, specific attention must be paid to proportions at the detection limit of 30 and 50%

It can be concluded that (Figure 2.1):

- to obtain a statistically significant difference for 30% from 40% at least 356 individuals must be tagged in one tagging event;
- to obtain a statistically significant difference for 50% from 40% at least 388 individuals must be tagged in one tagging event.

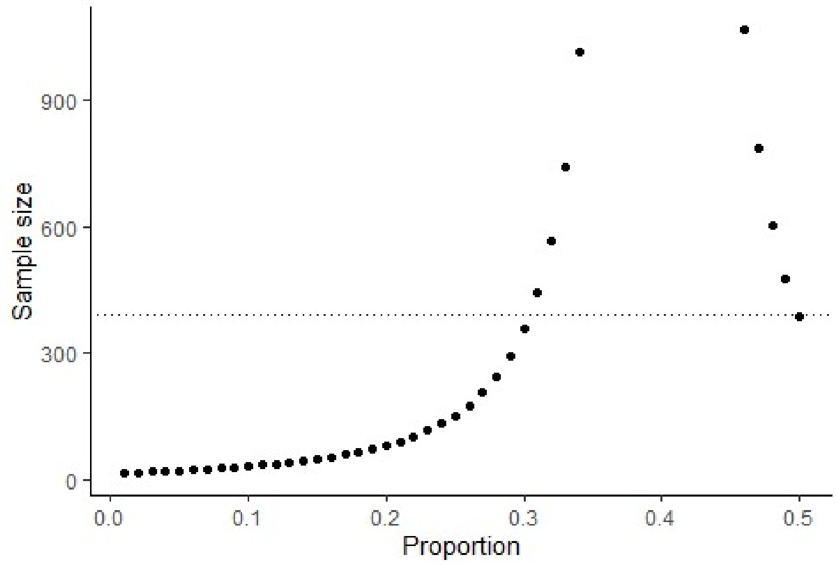

Figure 2.1 Change of sample size necessary to acquire statistically significant difference from 40% with 95% confidence and 80% power.

Decrease in sample size not only increases the detectable distance from the 40% fishing pressure but also lowers the power of the test. The results of analysis of test power to distinguish statistically significant differences at 30% and 50% from 40% with 95% confidence confirms that the minimal sample size for a particular mark-recapture survey is 388 specimens (Figure 2.2). Smaller sample size will greatly decrease the power of the test and also increase the distance from 40% which can be detected.

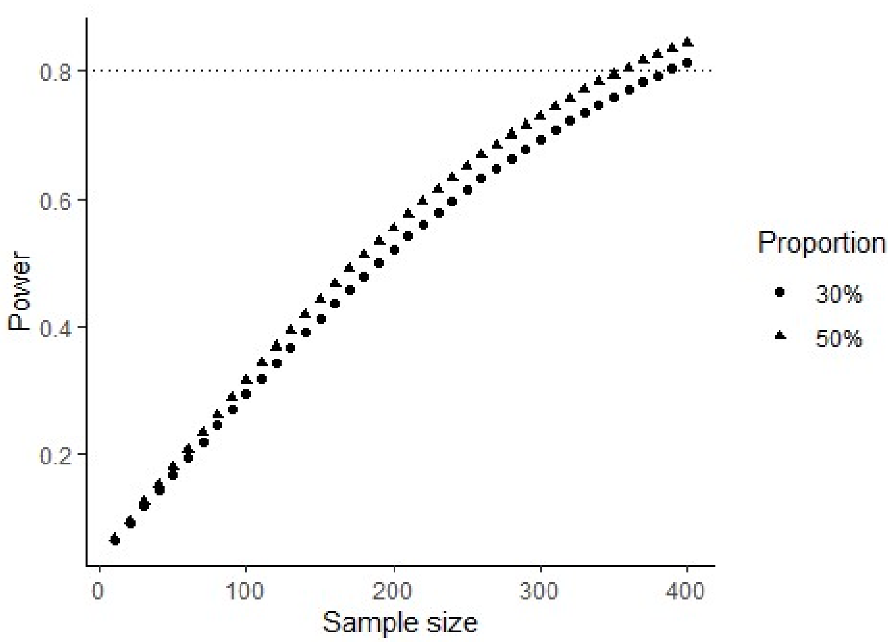

Figure 2.2 Change of the power of the test with increasing sample size

During the LAMPREY project specimens tagged for mark-recapture survey were released in the river of interest. However later noteworthy part of them emigrated to the sea and entered other rivers in such way complicating the evaluation of the results. It must also be taken into account that the results of the mark-recapture study from one particular river will be hard to extrapolate to all region. Therefore, we recommend to release the tagged specimens in the sea. It is important that the release of tagged specimens imitate natural river lamprey spawning run as closely as possible. On the other hand, it is self-evident that a complete mimicking of natural migration is not possible. Performing of the mark-recapture surveys is also limited by the length of fishing season that restricts both acquisition of lampreys for tagging and recapture of tagged specimens. The project region includes a great part of Latvian coastline both in the main proper of the Baltic Sea and the Gulf of Riga, therefore, places of release of tagged specimens should include both of these water bodies. Our previous experience with the release of tagged lampreys in the Eastern and Southern part of Gulf of Riga suggests that after the release in seashore the tagged lampreys tend to enter the river closest to the place of release or move to the greatest river in the proximity of release point. In Western part of the Gulf of Riga, lamprey fishing takes place in River Roja and in three smaller streams – River Grīva located southwards and rivers Pilsupe and Melnsilupe located northwards of the River Roja. This suggests that in the Eastern part of the Gulf of Riga tagged specimens should be released in at least two sites – northwards and southwards of the River Roja. In main proper of the Baltic Sea, lamprey fishing in Latvia is carried out in River Venta - the largest river in the region, River Irbe - located northwards and rivers Užava, Rīva and Saka located southwards of River Venta. River Saka is the second most important river for lamprey fisheries after River Venta. This suggests that the specimens tagged in Latvian coastline of the Baltic Sea main proper should be released in at least three points: between rivers Venta and Irbe, between rivers Venta and Užava and between rivers Saka and Rīva. Most of the Lithuanian rivers flow into Curonian lagoon which is connected to the Baltic Sea main proper with relatively narrow straight next to Klaipėda, however, River Šventoji where lamprey fishery is carried out, as well as several smaller rivers with no lamprey fishery empty in the main proper of the Baltic Sea. Therefore, at least two release sites are recommended for Lithuania – northwards and southwards of River Šventoji. Third additional release site inside the Curonian lagoon should be considered as well. In both countries, sites of release should be stationary.

As mentioned above, the possibility to successfully perform a mark-recapture study is limited not only by lamprey migration but also by the fishing season. In rivers Venta and Saka fishing season starts on August 1st and in the rest of Latvian rivers – on November 1st. In Lithuania fishing season starts on October 1st and in both countries fishing season lasts until the end of January of the following year. In all release sites lampreys should be tagged at least twice in November targeting to perform one of the tagging actions shortly before and one – just after the peak intensity of migration. Entrance of lamprey spawners into the rivers is facilitated by the increase of river discharge and onshore winds, and it is slowed by the increase of illumination from the moon (Ryapolova, 1964; Evtjuhova, Ryapolova, 1967; Aronsuu, 2015; Abersons, Birzaks 2014). In addition, one additional release of tagged specimens should be performed in August or September in the three release points within the Baltic Sea main proper in the territory of Latvia.

In studies prior to this project, different types of tags have been used for lamprey tagging in Latvia, including self-locking "cinch-up" loop tags, spaghetti tags, streamers and other. In general, all types of tags proved to be convenient for performing mark-recapture studies. As a result, the most important aspect for choosing the type of tag for further use is the swiftness of the tagging process itself. The faster the process of tagging is, the smaller the impact it has on the lamprey tagged. In terms of speed the streamer tags are the undisputable leader from all the tag types we have used in previous studies. Speed of tagging can be increased even further if streamer tags are packed on field-ready cards in sequential order. To reduce the impact of tagging, the tags should be inserted under the skin in a fat layer before the dorsal fin. Information printed on the tag should be sufficient to identify when and where each specific lamprey was tagged and released. The easiest way is to use tags with unique printed-on numbers and register which tags were used in each tagging session. There are several additional options such as using different types of tags or differently coloured tags etc., however, we believe that such approach would complicate the monitoring and suggest avoiding it.

Previous mark-recapture and other studies confirm that lampreys can easily survive without water for at least one hour, and specimens for tagging can be kept in dry open-top plastic boxes. However, additional stress can impact the behaviour of the animal and therefore handling of lampreys should be as gentle as possible. To reduce the impact of treatment, it is recommended to keep lampreys in two or more mesh bags submerged in natural waterbody during the tagging and remove them from water only for the time needed for insertion of the tag and during the transportation. Lampreys are also vulnerable to freezing and drying out in the sun. Therefore, it is important to protect lampreys from freezing or direct sunlight as much as possible both during the tagging and transportation.

To be able to utilize the classic mark-recapture data analysis methods (known-fate analysis, Cox PH, etc.), it is necessary to not only know that the lamprey has been recaptured, but also to obtain the accurate information about the location and time of the recapture. So, the success of all study greatly depends on the quality of information provided by the fisherman who are the first and often also the only individuals that come into contact with the fishing gear and captured lampreys. During the LAMPREY project most of lamprey fishermen in Latvia were asked to fill in a questionnaire regarding the noticing and reporting the capture of the tagged lamprey. 57% of fishermen confirmed that marked lampreys are relatively easy to spot in their catch, however, 32% indicated that large catch size is an obstacle for noticing marked lampreys. Another fact that confirms potential problems in noticing marked lampreys is that only 11% of fishermen noticed them primarily in fyke-net. 32% of fishermen said that the marked lampreys were noticed in both fyke-net and storage box, and 57% noticed them only in the box. Storage boxes are boxes with holes submerged in a river where captured lampreys are stored during the period when demand in market or the market price for lampreys is too low. Most fishermen could not name precise measures that would increase their interest in inspecting their catch more carefully and become more active in reporting of tagged lampreys (53%), or they indicated that such measures are not necessary (11%). However, 25% of the surveyed fishermen answered that monetary rewards could increase their interest, and 11% said that more extensive information about the research, its process and goals would be sufficient motivation to participate. The experience gained in this project as well as previous studies demonstrate that fishermen are in fact more interested in providing information about recapturing of tagged specimens if regular communication is provided and interest is shown on a personal level. Even though monetary rewards and communication on daily basis could be effective methods for obtaining more accurate information, it is important to keep in mind that either of these options would significantly increase the effort necessary to carry out the survey.

2.2 Guidelines for monitoring of the upstream migrating adult river lampreys

2.2.1 Method and equipment

Recommended method for monitoring of the adult river lamprey migrating upstream is the mark-recapture survey in cooperation with a commercial fisherman. Lampreys for mark-recapture study should be purchased directly from fisherman and fisherman need to also be involved in reporting of capture of tagged lampreys.

The uniquely numbered streamer tags packed in the field-ready cards in the sequential order have proved themselves to be the most convenient equipment for tagging the river lamprey. However, any reasonably sized external tag which allows identifying when and where the exact specimen was tagged and released is acceptable.

2.2.2 Sample size and release sites and timing for release of tagged lamprey

The recommended minimal sample size in one tagging event is 388 individuals. Tagged lampreys should be released in the sea in proximity of the seashore. In Kurzeme Region in Latvia the tagged lamprey needs to be released in at least 5 sites and in Lithuania there are 2 mandatory release sites and 1 additional optional site. Recommended release sites for Latvia are southwards and northwards from River Roja, between rivers Venta and Irbe, between river Venta and Užava and between rivers Saka and Rīva. Recommended release sites in Lithuania are southwards and northwards of River Šventoji (mandatory) and inside the Curonian lagoon (optional) (Figure 2.3.). In all sites the tagging and release should be performed twice in November – one time before the peak of spawning migration and one time after this. In addition, in three Latvian sites located in the Baltic Sea additional tagging should be done in August or September. All sites should be stationery i.e., the release of tagged lampreys should be done in exactly the same place as previously.

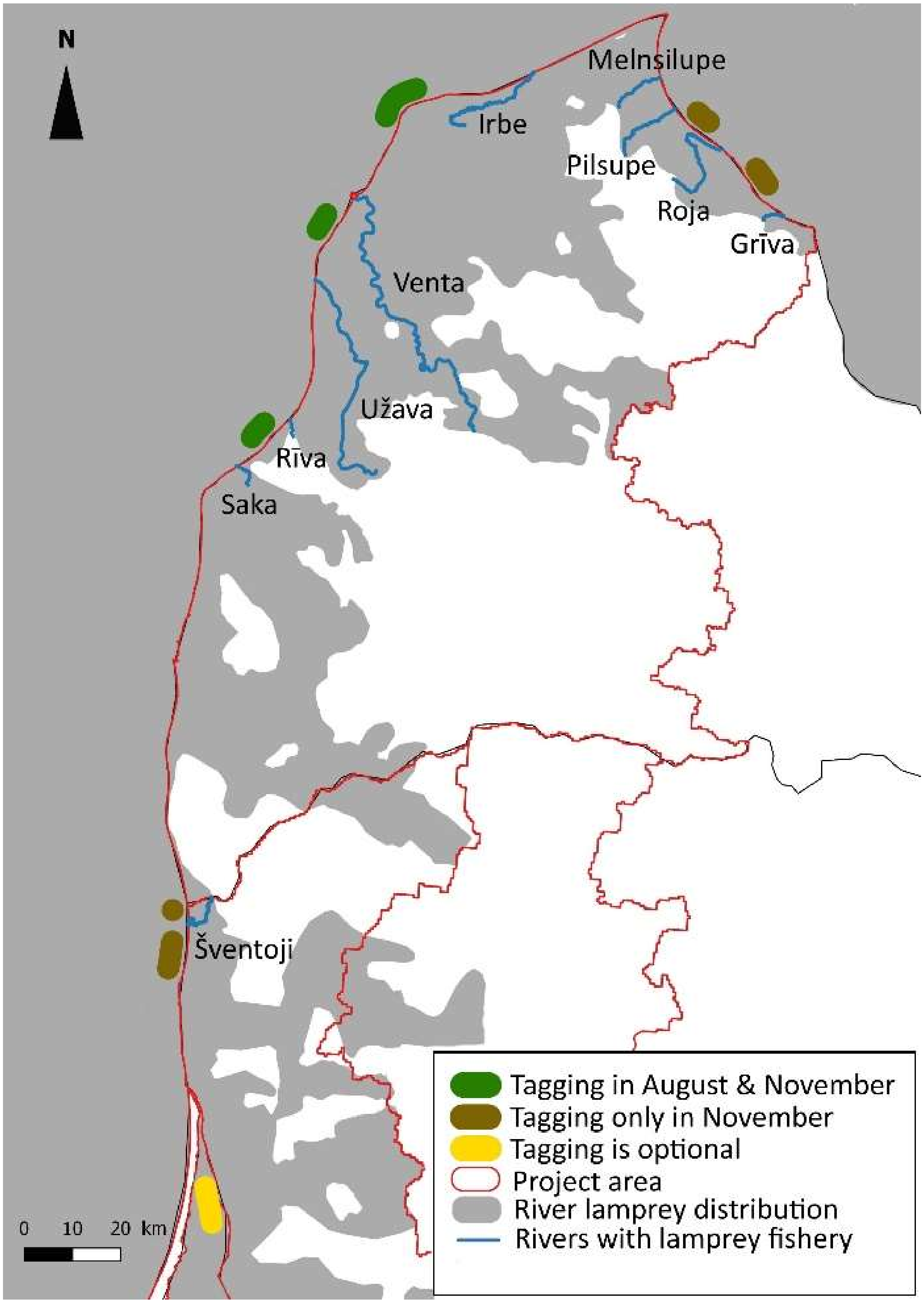

Figure 2.3. Recommended location of sites for release of tagged lampreys and timing for tagging

2.2.3 Handling of lampreys and tagging procedure

Tags should be inserted under the skin in a fat layer before the dorsal fin (Figure 2.4). The procedure of insertion of the tag can vary depending on the type of the tag and also on the producer of a certain tag. Therefore, we recommend contacting the producer of tags used and follow their instruction.

Figure 2.4. River lamprey tagged with a streamer tag.

Handling of the lampreys should be as careful and swift as possible. If possible, lampreys should be stored in the water (preferably in a mesh bag placed in a natural waterbody or in the oxygenated tank) and removed from a water for as short time as possible. It is recommended to confine the time outside the water only to the time needed to insert the tag. However, if it cannot be avoided, lampreys can also be stored in dry boxes for a time that does not exceed 30 min. **NB! avoid exposing lampreys to the freezing temperatures and direct sunlight as much as possible.**

2.2.4 Collecting information on recapture and data analysis

Collection of recapture information should be done in cooperation with commercial fishermen. It is important that besides the information on recapture of tagged lampreys, the information on exact time and place of recapture is also collected. To facilitate the participation of fishermen all of them should be contacted and encouraged to cooperate in the beginning of fishing season, a few days before tagging and every week after tagging. It is recommended to increase the involvement of fishermen by small monetary rewards for returned tags.

Method for data analysis greatly depends on the quality of data which cannot be prognosed precisely. If possible, we suggest the usage of already existing methods for analysis of mark-recapture data such as known-fate analysis, Cox PH or other. If the available data does not allow the use of such methods the data analysis should be carried out using any other convenient method.

3. Guidelines for monitoring of the efficiency of implemented river lamprey stock maintenance measures

3.1. Background information and rationale

Despite the relatively large experience of stocking lamprey larvae and other measures for maintaining river lamprey population, the efficiency of these measures is still largely unknown. To increase our knowledge of success of different measures and to identify the possibility of increasing their efficiency convenient monitoring is needed. It is expected that the majority of mitigation measures (stocking of larvae, translocation of spawners, facilitation of migration etc.) will be implemented in freshwater and aimed at increasing the abundance of lamprey larvae. Therefore, the most suitable method for monitoring of the success of implemented maintenance measures is the monitoring of lamprey larvae. Such monitoring plays only a supplementary role in the maintenance projects and the costs for the monitoring must be as low as possible. To reduce both the effort and costs it is important that the field work needed for evaluation of one sampling site can be finished within a day.

Test power in count data analysis mostly depends on lambda of Poisson distribution (the larger the population, the easier it is to detect its change) and number of replicates (the more information is available, the more accurate the measurement is, if not inflated by zeros) and the effect size itself. In the monitoring of success of transportation of lamprey spawners above Ventas Rumba waterfall the relative risk for age group 0+ was centred at 4 and 5 with 95% confidence minimum at 1.6 and 1.9 respectively. Results of the simulation trial suggest that at least eight samples (i.e. sapling sites with compound number of individuals from individual samples) are needed to detect population doubling with lambda 3 (Figure 3.1).

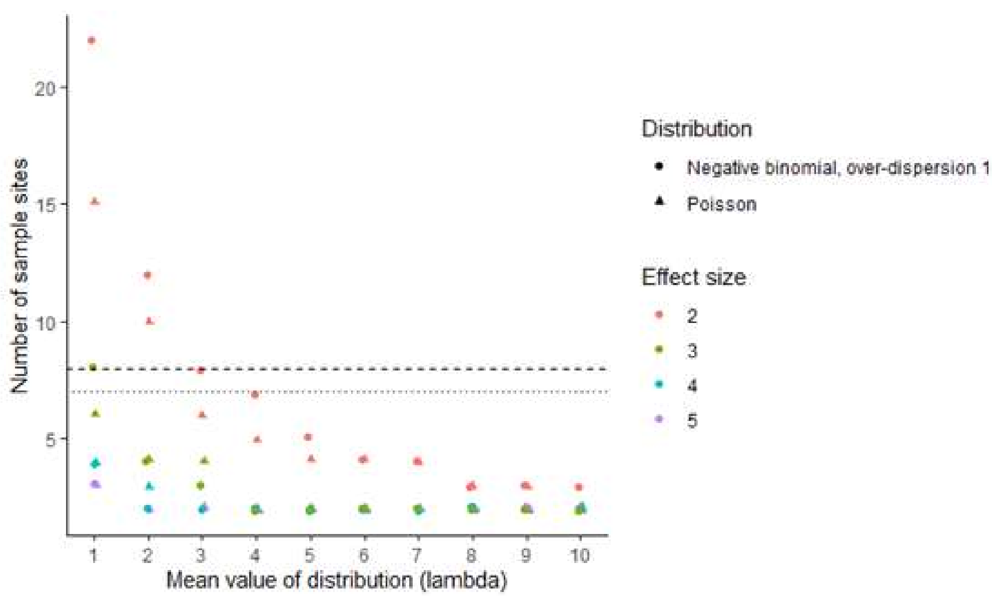

Figure 3.1. Relation between the effect size and lambda in two distributions with number of samples needed to detect statistically significant population changes (80% power and 95% confidence). Dotted line – seven, dashed line – eight sampling sites.

Average density of lamprey larvae before translocation of spawners upstream the Ventas Rumba waterfall was from 0.6 to 0.9 individuals per square meter. Assuming that the average situation in other rivers is similar, it can be concluded that at least 5 square meters should be sampled to acquire lambda = 3. Area of the box used for sampling of lamprey larvae is 0,12 m^2 (see chapter 1.1 and Lasne *et al.* 2010 for more information). Therefore, at least 25 random samples would be needed in one sampling site. However, this means a total of 200 (25 x 8) bottom samples which is approximately twice of the workload for one day and other solution is needed. The lambda can be augmented not only by increase of area of sampling site but also if random sampling is replaced by targeted sampling. Maintenance measures should be carried out in rivers with very week or with no lamprey population and it is expected that larvae will first occupy the most suitable habitats. We assume that the targeted sampling in optimal habitats will allow to reach expected lambda using smaller number of samples. Analysis of annual lamprey monitoring data confirms that the greatest abundance of lamprey larvae usually is found in bank-related riverbed formations (coastal slope of riverbed or small bays of coastline) in 35-55 cm depth with no human disturbance and soft (dominated by sand and detritus) riverbed (see Figure 1.4 in Chapter 1.1). Average expected density of lamprey larvae in such habitats is 10 to 13 larvae per square-meter (95% Cl). Therefore, in average only 4 samples (total sampled area 0.48 m^2) will be needed to capture at least 4 lamprey larvae, at least 4 samples. However, it also must be taken into account, that most of the calculations are based on the monitoring data which is collected mainly in rivers with strong lamprey population and in river reaches that are suitable for lamprey larvae. Therefore, we suggest to increase the number of samples in one sampling site to five. Also, a larger number of the sampling sites is needed to provide more accurate generalisation for efficiency of the implemented measures. As the maximum workload for one day is approximately 100 samples, we suggest to increase the number of sampling sites to 16–20, depending on the size and other parameters of the river. Bottom sampling can also be carried out by the lamprey sampling shovel.

In Latvia this shovel was used for lamprey monitoring until 2018 and its suitability for lamprey monitoring was also proved during intercalibration of Latvian and Lithuanian methods performed within LAMPREY project (see Figure 1.3. in Chapter 1.1). However, it is relatively hard to estimate the precise area for each sample of the shovel and this at some extent will compromise the data quality. Therefore, we recommend to use the shovel only if the use of sampling box is not possible due to the rock-dominated bottom or other reasons. The area of a single sample for shovel is smaller than that for the lamprey sampling box therefore the larger number of samples is needed. If sampling is done by the shovel, we suggest taking at least 7 samples (sampled area ~0.42–0.49 m^2) in one sampling site.

Sampling must be performed at least two times – before implementation of the maintenance measures and after these measures are implemented. However, we suggest to continue the monitoring for a longer period, if possible, for four years after the implementation of measures. The sampling gear as well as location of sampling sites and number of the samples taken in each site should be the same during all period of monitoring.

Handling of captured larvae in general must be the same as described in Chapter 1.2.3., i.e., captured larvae should be sedated in clove oil, measured to nearest mm and released back in the river after recovery. In each sampling site the GPS coordinates of the site and size of lamprey larvae captured in each sample need to be registered. Captured lampreys must be divided in three age groups (subyearlings or 0+, yearlings or 1+ and older) as described in chapter 1.1. Data on each age group can later be used for both TRIM analysis (as a sum of samples per area) or as independent samples within generalized mixed effects modelling (with samples nested by sampling area).

Other methods such as e-DNA analysis or electrofishing can be used as well. Use of these methods is suggested in rivers or parts of rivers where the lamprey population did not exist or was exceptionally week before the implementation of maintenance measures. In such rivers the success of maintenance measures can be confirmed by detecting the existence of the lamprey population as such and therefore the use of the labour consuming methods for estimation of the abundance of lamprey larvae is not required. E-DNA sampling and electrofishing should be performed in line with already existing protocols and standards.

3.2 Guidelines for the monitoring of efficiency of the maintenance measures

3.2.1. Method

Suggested primary method for monitoring of success of lamprey population maintenance measures is bottom sampling with lamprey box (Figure 1.1 in Chapter 1.1, for more information see this chapter and Lasne *et al.* 2010). Optional method is the use of bottom sampling shovel (Figure 1.2 in Chapter 1.1., for more information see this chapter), however use of the shovel is acceptable only if due to substratum of rivered or other specific condition the use of sampling box is not possible.

In specific cases when the maintenance measures are implemented in rivers with exceptionally weak or without any lamprey, the success of these measures (i.e., occurrence of noteworthy lamprey population) can be confirmed also by e-DNA survey or by electrofishing. E-DNA testing and electrofishing should be performed in line with already existing protocols and standards.

3.2.2 Sampling sites, samples and timing for sampling

Smallest acceptable number of sampling sites is 16 and smallest acceptable number of samples in one site is 5. However, when possible a larger number of sampling sites or samples is welcomed.

The number and location of sampling sites as well as the number of samples in each site should be the same during all monitoring period. Location of sampling sites must be selected for all rivers individually by taking into account the length and other parameters of the specific river and its accessibility. Sampling should be done in August or September.

3.2.3 Process of sampling

If possible, samples should be located in coast-related formations or riverbed-related formations (see Chapter 1.1). Before sampling one must consider the sampling strategy in each site in order to identify the potentially most suitable habitats and to avoid disturbing them while taking other samples. The sampling procedure is the same as described in Chapter 1.2.3 (i.e., the bottom sample must be washed through the mesh and captured lampreys collected, sedated in clove oil, measured to the nearest mm and after recovery released into river). However much less information than in "regular" monitoring of lamprey larvae described in Part 1 of this document has to be collected during sampling.

Only information which has to be registered is the GPS location of the sampling site and the length of lamprey ammocoetes (separately for each individual sample). Due to minimal amount of information to be registered the specific form for data registration is not needed.

3.2.4 Data analysis

Captured lampreys must be divided in three age groups (subyearlings or 0+, yearlings or 1+ and older) as described in chapter 1.1. Data on each age group separately can later be used for both TRIM analysis (as a sum of samples per area) or as independent samples within generalized mixed effects modelling (with samples nested by sampling area). If necessary, the results from "regular" monitoring of lamprey larvae can be used as a control.

References

Abersons, K., Birzaks, J., 2014. River lamprey, Lampetra fluviatilis L., fishery in Latvia – insight into the origin of catch statistics data. Archives of Polish Fisheries, 22, 169–179

Aronsuu, K., Marjomäki, T.J., Tuohino, J., Wennman, K., Vikström, R., Ojutkangas, E., 2015. Migratory behaviour and holding habitats of adult river lampreys (Lampetra fluviatilis) in two Finnish rivers. Boreal Environ. Res. 20, 120–144

Eglīte R. 1961 – Migration and spawning of river lamprey Lampetra fluviatilis (L.) in the rivers of the Latvian SSR – P. Stučkas Latvijas Valsts Universitātes Zinātniskie raksti 39: 9-28 (in Latvian)

Evtjuhova B., Ryapolova N., 1967 – The impact of hydrometeorological factors on the dynamics of spawning migration of Atlantic salmon and river lamprey – In: Rybohozjaistvennye issledovanija v bassejne Baltijskogo morja, Zvajgzne, Riga 2: 53-65 (in Russian)

Dalgaard, P., 2002. Introductory Statistics with R 2nd edition. Springer, New York. doi: 10.1007/978-0-387-79054-1

Lasne E, Sabatié M-R, Tremblay J et al (2010a) A new sampling technique for larval lamprey population assessment in small river catchments. Fish Res 106:22–26

Moser M.L., Butzerin J.M., Dey D.B. 2007. Capture and collection of lampreys: the state of the science. Reviews in Fish Biology and Fisheries 17 (1): 45–56

Pannekoek, J., Strien, A. Van, 2005. TRIM 3 Manual (Trends & Indices for Monitoring data). Statistics 1–58

R Core Team, 2019. R: A language and environment for statistical computing

Ryapolova N., 1964 – About the Consequences of spawning migration of River Lamprey *Lampetra fluviatilis* L. in the Rivers of the Latvian SSR – In: Trudy molodyh uchonyh, Pishhevaja promyshlennost', Moscow: 66-69 (in Russian)

Soldaat, L.L., Pannekoek, J., Verweij, R.J.T., Turnhout, C.A.M. Van, Strien, A.J. Van, 2017. A Monte Carlo method to account for sampling error in multi-species indicators. Ecological Indicators 81, 340–347. doi:10.1016/j.ecolind.2017.05.033

Appendix. Suggested data registration form for lamprey larvae monitoring

1. Overview of the River Section

№ _____ DATE_________________ RIVER_________________________ CATCHMENT______________________

PLACE___________________________________ COORDINATES___________________/____________________

	Depth < 35 cm	Depth 35-55 cm	Depth > 55 cm	Width, m	Macrophyte coverage, %
River					
Sampling plot					

Macrophyte coverage, %: algae____,nympheids____, elodeids _____, helophytes_____, lemnids______

In the sampling plot, %: algae_____,nympheids_____, elodeids______, helophytes______, lemnids_____
(Section "in the sampling plot" must be filled only if the sampling plot isn't all across the river)

Nympheids – leaves and blossoms on the water surface (*Nymphoides*, *Nuphar*, *Saggitaria*, *Sparganium*, *Potamogeton*); **Elodeids** – fully underwater except for the blossoms (*Elodea*, *Myriophillum*, *Potamogeton*) **Helofphytes** – only the bottom of plants body is under water (*Phragmites, Typha, Sparganium*); **Lemnids** – floating on water surface (*Lemna*, *Hydrocharis*) **Note: the category of waterplants sometimes depends on the emplacement of leaves in the water, e.g. *Sparganium or Potamogeton***

Stream velocity category: _______
0 – there is no visible water movement neither in sampling plot, nor within sight; **1** – if in the sampling plot or within sight water flows, but does not exceed 0,2 m/s; **2** – if within sight there is a section, where stream velocity exceeds 0,2 m/s; **3** – if stream velocity exceeds 0,2 m/s **before and after** sampling plot, but within sight are also slower sections; **4** – if stream exceeds 0,2 m/s everywhere or almost everywhere.

Tributary (in the sampling site or upstream of it, if there is one), width m______

Anthropogenic impact, %

	Activity		Digging		Object		Migration obstacles		Beaver dam	
	Recent	Long ago	Recent	Long ago	Recent	Long ago	Distance to dam, m	Distance to HPP, m	Above, dist, m	Below dist, m
River										
Sampling plot										

Activity – place disturbed by wading, boating, swimming etc. (recently – if the marks of boats, people, cars are visible; long ago if there are no visible marks of recent activity); **Digging** – digging, melioration or other significant mechanical change of riverbed (recently, if the biotopes are determined by modifications; long ago, if the riverbed has already +/- modified back to normal) **Object** – bridge, embankment etc. (recently, if the marks of construction activities are visible in riverbed; long ago, if only the object itself is visible); **Migration obstacles** – dam, weir, waterfall or other obstacle, that has significant impact on lamprey migration, mark if it is hydro power plant or other, or both, and the distance to it, in meters (from databases and/or maps) ; **Beaver dam** – if within sight

DOMINATING SUBSTRATUM CATEGORY (**only one** of the categories – if the sampling plot is all across the river, mark only in "river", if not then also in "sampling plot"):

RIVER		SAMPLING PLOT	
Unsuitable Clay, dolomite, etc.		Unsuitable Clay, dolomite, etc.	
Stone Diameter at least 20 cm, share of stones exceed 50%		Stone Diameter at least 20 cm	
Mixed, hard Stone + at least 50% sand or gravel		Mixed, hard Stone + at least 50% sand or gravel	
Sand and gravel Gravel at least 5%		Sand and gravel Gravel at least 5%	
Soft Detritus, sand etc. (gravel <5%, stone up to 30%)		Soft Detritus, sand etc. (gravel <5%, stone up to 30%)	

RIVERBED HETEROGENEITY IN THE SAMPLING PLOT:
Mark proportion % ONLY in that river part, where sampling will be done:

Coast-related-heterogeneity_________________
Curves, coast and pit slopes, that are related to coastline

Riverbed-related_________________
Silts before/after/around obstacles, pit beginnings and ends

Big formations__island/ peninsula__________
Bay in coast side of peninsula, silts after islands, mark if one of them

In the stream_________________
+/- straight riverbed, without obvious formations

pH: ___________

Conductivity, µS/cm: _____________

Dissolved oxygen, mg/L: _________

T^{o}, C: _______

Time of photo: ___________

2. Characteristics for each sample

№	Formation		Depth, cm*		Slope**		Macroph. cov, %		Anthrop. impact		Substrate, %	
1	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
2	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
3	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
4	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
5	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
6	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
7	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
8	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
9	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
10	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
11	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	
12	Coast-rel		<35		0		Algae		Yes		Organic	
	Riverb.-rel		35-55		1		Elod				Sand	
	Big form.		>55		2		Nimph		No		Gravel	
	Stream				3		Heloph				Stone	

*precise depth must be noted

** **0** – horizontal or a little sloping, **1** – sloping, **2** – sloping a lot, **3** – vertical or almost vertical

3. The Table of Ammocoetes

Sample №	Larva №	Length, mm	Notes	Sample №	Larva №	Length, mm	Notes

Sample №	Larva №	Length, mm	Notes	Sample №	Larva №	Length, mm	Notes

4. River characterization by transect method

		Transect №*										
		1	2	3	4	5	6	7	8	9	10	11
		1 m										
	Depth, cm											
	Stream, m/s											
Mineral. substr. Sum=100%	>20 cm (stones)											
	2-20 cm (pebble)											
	0,2-2 cm (gravel)											
	<0,2 cm (sand)											
Org. Sum ≠ 100	Detritus (coarse)											
	Detritus (fine)											
Macrophytes code**, sum≠5	Algae											
	Helophytes											
	Nympheids											
	Elodeids											
	Lemnids											
		3 m										
	Depth, cm											
	Stream, m/s											
Mineral. substr. Sum=100%	>20 cm (stones)											
	2-20 cm (pebble)											
	0,2-2 cm (gravel)											
	<0,2 cm (sand)											
Org. Sum ≠ 100	Detritus (coarse)											
	Detritus (fine)											
Macrophytes code**, sum≠5	Algae											
	Helophytes											
	Nympheids											
	Elodeids											
	Lemnids											
		5 m										
	Depth, cm											
	Stream, m/s											
Mineral. substr. Sum=100%	>20 cm (stones)											
	2-20 cm (pebble)											
	0,2-2 cm (gravel)											
	<0,2 cm (sand)											
Org. Sum ≠ 100	Detritus (coarse)											
	Detritus (fine)											
Macrophytes code**, sum≠5	Algae											
	Helophytes											
	Nympheids											
	Elodeids											
	Lemnids											

		7 m										
	Depth, cm											
	Stream, m/s											
Mineral. substr. Sum=100%	>20 cm (stones)											
	2-20 cm (pebble)											
	0,2-2 cm (gravel)											
	<0,2 cm (sand)											
Org. Sum ≠ 100	Detritus (coarse)											
	Detritus (fine)											
Macrophytes code**, sum≠5	Algae											
	Helophytes											
	Nympheids											
	Elodeids											
	Lemnids											

		9 m										
	Depth, cm											
	Stream, m/s											
Mineral. substr. Sum=100%	>20 cm (stones)											
	2-20 cm (pebble)											
	0,2-2 cm (gravel)											
	<0,2 cm (sand)											
Org. Sum ≠ 100	Detritus (coarse)											
	Detritus (fine)											
Macrophytes code**, sum≠5	Algae											
	Helophytes											
	Nympheids											
	Elodeids											
	Lemnids											

*Each of the transect must be done in ~2 m distance from the previous one.

Nympheids – leaves and blossoms on the water surface (*Nymphoides*, *Nuphar*, *Saggitaria*, *Sparganium*, *Potamogeton*); **Elodeids** – fully underwater except for the blossoms (*Elodea*, *Myriophillum*, *Potamogeton*) **Helofphytes** – only the bottom of plants body is under water (*Phragmites, Typha, Sparganium*); **Lemnids** – floating on water surface (*Lemna*, *Hydrocharis*) **Note: the category of waterplants sometimes depends on the emplacement of leaves in the water, e.g. *Sparganium or Potamogeton***

****Code:**

+ = <1% **1** = 1-5% **2** = 5 - 25% **3** = 25-50% **4** = 50-75% **5** = 75-100%