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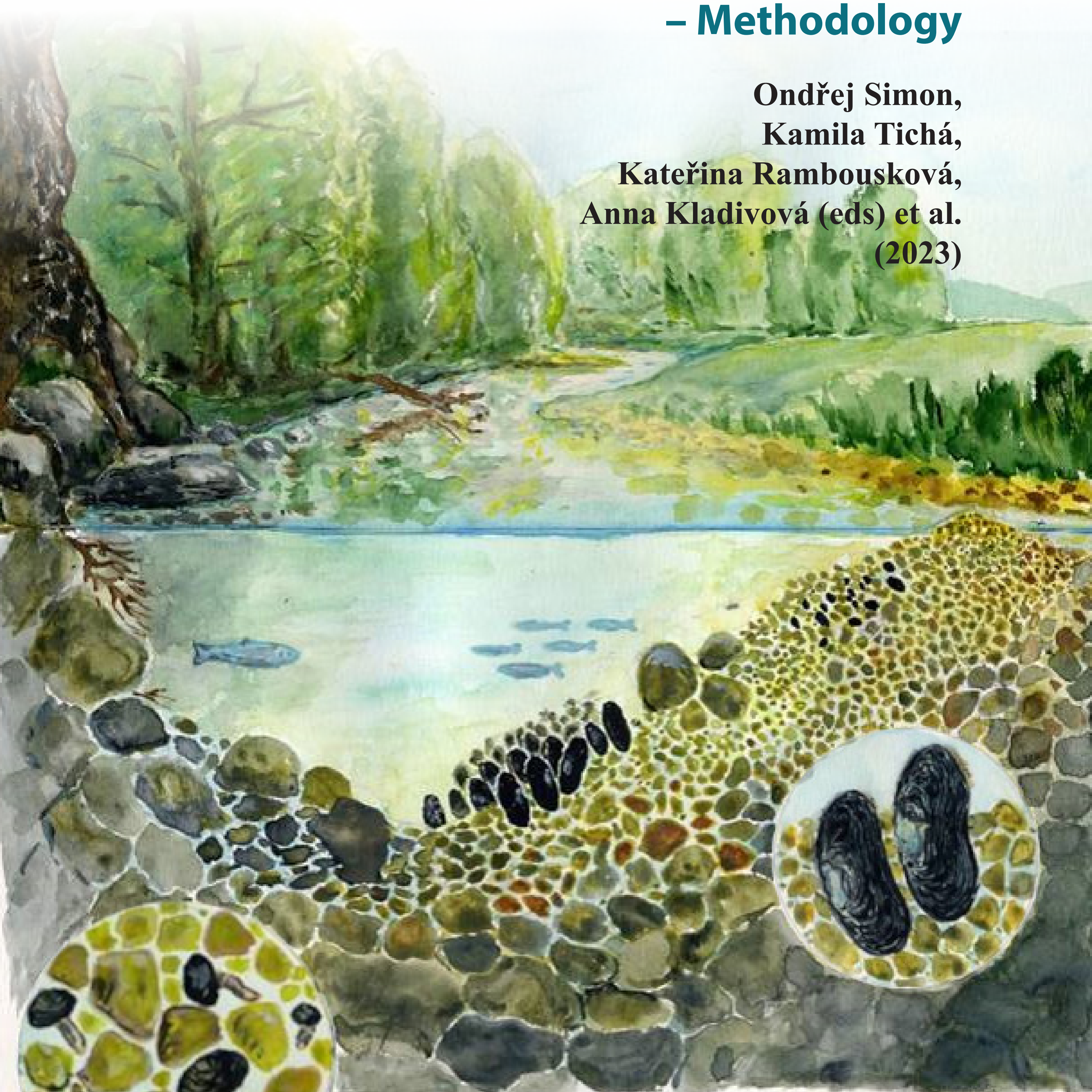
Supporting freshwater pearl mussel (*Margaritifera margaritifera*) – Methodology

Ondřej Simon,
Kamila Tichá,
Kateřina Rambousková,
Anna Kladivová (eds) et al.
(2023)



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T. G. Masaryk Water Research Institute
Podbabská 2582/30, 160 00 Praha 6,
Czech Republic
IČ: 00020711
+420 220 197 111
www.vuv.cz

Faculty of Environmental Sciences
Czech University of Life Sciences Prague
Kamýcká 1176, 165 00 Praha 6, Czech Republic
IČ: 60460709
+420 224 384 351
www.fzp.czu.cz

Authors:
Michal Bílý
Michaela Černá
Bohumil Dort
Jitka Horáčková
Jaroslav Hruška
Anna Kladivová
Věra Kladivová
Kateřina Rambousková
Ondřej Simon
Jan Švanyga
Kamila Tichá
Alena Vydrová

Expert reviewers:
Tomáš Bodnár
Dagmar Brejšová
Ivana Bufková
Karel Douda
Jaroslav Hruška
Tomáš Just
Michala Mariňáková
Robert Ouředník
Ondřej Spisar
Jan Švanyga

The author of the drawing on the front cover is Michal Bílý.
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Prologue

It has been almost 40 years since Miroslav Hána, the former head of Šumava Landscape Protected Area, contacted the Czech Union for Nature Conservation in Volary in order to participate in freshwater pearl mussel (*Margaritifera margaritifera*) protection. In those days 130 000 adult mussels lived in the Blanice River upstream from Husinec reservoir. Adult mussels were continuously covering the river bottom at some sites; however, natural reproduction had been stagnated over 20 years.

Invited experts from abroad were impressed when visiting this locality and they saw no reason for reproduction problems. Water of good quality, suitable fish stock, optimal structure of the river bed in many places. In addition, mostly uncultivated river floodplains and relatively low settlement. All this seemed to guarantee good function of the pearl mussel habitat. In most Central European localities, the main cause of the extinction of pearl mussels was excess nutrients – eutrophication. It was necessary to look for hidden causes, and it really was not an easy job.

It was necessary to involve experts from different professions, monitor all events in the watershed, establish experimental areas, and observe the effect of different management activities on habitat function. Gradually, this research was extended to other Czech pearl mussel localities, declaration of seven protected areas was prepared, and other stream parts were included among the Special Areas of Conservation (SACs).

The main question – why young pearl mussels had hardly appeared for several decades – was finally answered by the pearl mussels themselves. Surprizing and unexpected findings were obtained from semi-natural rearing and thorough monitoring of the reactions of the youngest stages under natural and model conditions at experimental sites. Reproduction proceeds successfully until the juveniles leave the host fish. Then two options arise. First, the juveniles are in a stream with large organic production, where they cannot dig into the river bed because it is not oxygenated enough. They grow rapidly for some time, but they cannot create a sufficiently viable population in the altered habitat. The second possibility is that the juveniles are in a stream that used to be their historical habitat and does not seem to have changed. The water and river bed are clean. However, food supply has changed and there is not enough for the growth of the shells, which must be fast enough to prevent corrosion. Corrosion disrupts the calcium shell of bivalves and is natural in these streams. Something is different and this cannot be easily corrected.

It is remarkable that these long-lived bivalves were able to adapt to life in cold, very nutrient-poor waters that other animals could not use, and yet they formed massive colonies with huge biomass. Their natural area is located in watersheds with a calcium-poor geological base, although they necessarily need calcium for their massive shells. This could only be achieved by a food supply that does not originate in the flows, but in the vegetation and soils of the watersheds. This strategy was successful for centuries, until acidification reversed everything. This was not only long-distance transmission of pollutants, but also acidification of soils caused by changes in the composition of vegetation and farming methods.

We can feel the satisfaction that in the Czech Republic we have obtained significant knowledge from the biology of these critically endangered bivalves and know the main causes of reproduction stagnation in oligotrophic waters. We can enjoy first place in the development and practical implementation of rescue rearing of pearl mussels up to the fertility stage, in direct relation to their natural habitat. It may satisfy us that we have found ways to restore watersheds of pearl mussel rivers. However, we should be very worried that we are still unable to bring this knowledge to life in the necessary extent, while the remaining populations are disappearing before our eyes. That is why this methodology was written, to contribute to correcting the situation. Jaroslav Hruška, (2015)

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Chapter 1: Introduction and main goals

The freshwater pearl mussel (FPM) (*Margaritifera margaritifera*) is a critically endangered species in the Czech Republic, protected by both Czech and European law. According to Nature Conservation law (Government Decree No. 114/1992 Coll.), protection of FPM is guaranteed by species protection as well as landscape protection (The Natura 2000 protected areas network). Since 1999 there has been an Action Plan for Freshwater Pearl Mussel (CAP FPM) in the Czech Republic (Absolon & Hruška 1999), currently operated by the Nature Conservation Agency of the Czech Republic (NCA CR). The last update of CAP FPM was in 2013 (AOPK ČR 2013a).

Due to the insufficient impact of action plans and national legislation and conservation strategies in FPM population enhancement, there is also the need to involve local stakeholders such as foresters, farmers, landowners, and fishermen as well as local municipalities. Common good practice in the catchment areas and local stakeholder involvement are the main goals of this methodology.

FPM is a critically endangered mollusc strictly inhabiting oligotrophic watercourses with low nutrients concentration. Despite the former widespread distribution in the Czech Republic, there remained just few submontane rivers (**Fig. 1**) in Šumava Mountains, Novohradské hory Mountains, and Aš district border area till today. Not only water quality in the rivers but also the agricultural land use and forestry affect viability of Czech FPM populations. The high habitat quality demand of this stenoecious species is often in conflict with human intention but could be less. Better timing of particular human activities can reduce their negative impact on FPM sites.

The text below summarises and describes examples of the main environmental threats currently endangering FPM sites in the Czech Republic together with the Good Practice Handbook for stakeholders.

Fig. 1: Teplá Vltava River – an example of FPM natural habitat.

Chapter 2: Freshwater pearl mussel life cycle

Author: Ing. Kateřina Rambousková, Ing. Věra Kladivová, Mgr. Jan Švanyga, Mgr. Ondřej Simon
Reviewers: Mgr. Tomáš Bodnár, Robert Ouředník

The freshwater pearl mussel (FPM) has a very unusual and complicated reproductive period. It includes a parasitic larval stage requiring a host (**Fig. 2**). The FPM is a gonochorist (having just one of two distinct sexes in any one individual organism), but hermaphrodites can be found in low-density populations in the stream (i.e. having both sexes). In early summer months, males release sperm into the water column, while eggs are retained and fertilized inside the female (spermcast mating). Mussels can also undergo asexual reproduction and self-fertilize as hermaphrodites (Bauer & Wächtler 2001). Following the egg fertilisation, small larvae called glochidia develop in the female's brood chambers (specialized chambers in their gills). This period takes roughly four weeks (Hruška 1999) before the glochidia are released to the open water, which is usually in August. The process is cued by water temperatures where the thermal summation threshold must reach about 380–420 °C (Hruška

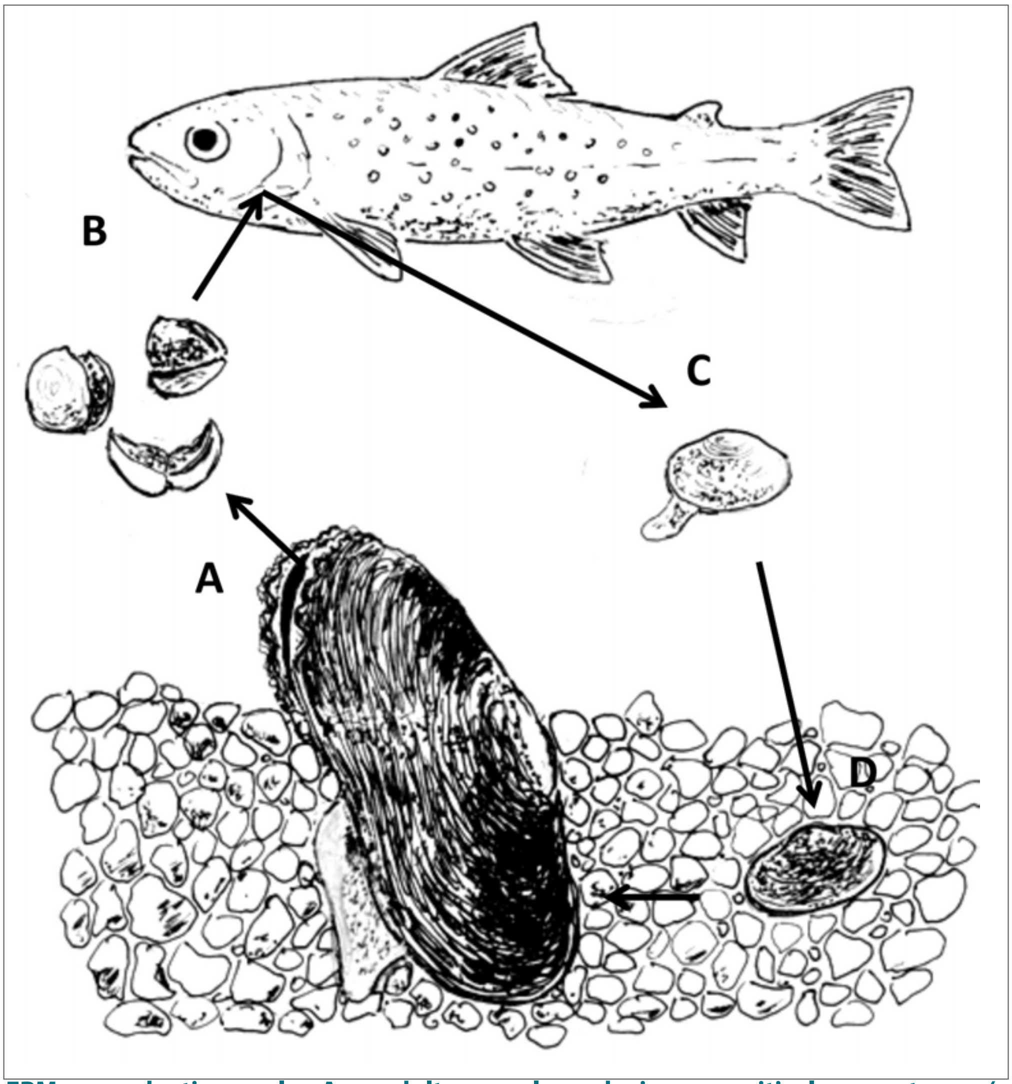

Fig. 2. FPM reproductive cycle. A – adult mussel producing parasitic larvae stages (glochidia), B – glochidia attach to host fish gills, C – after about one year, the juvenile falls off the host fish gills and buries into the river bottom (D). Illustration: Michal Bílý.

2000a). Then the glochidia are passively drifted short distances downstream of the sites from where they are released. Some of them meet their fish host and attach to its gills (Bauer 1988). This life cycle phase can be considered very critical as increased mortality of released larvae is typical for this stage. Salmo salar (Atlantic salmon) appear to be the main native host species of the FPM in Czech streams. However, after the construction of dams on the Labe River (especially Střekov in 1936), its migration completely stopped and now its occurrence in the Czech Republic is limited. Currently, Salmo trutta m. fario (river trout) is reported to be the only available host (Dyk 1992). Glochidia that successfully attach to the gills of a host fish become encapsulated by the host tissue, forming a cyst. They grow and develop until they reach the juvenile stage. The duration of the host-dependent phase is related to the water temperature at which they develop. Glochidial developmental phase of 11 months has been observed in South Bohemian streams. Once their metamorphosis has been completed, juvenile mussels excyst from the host tissue and drop to the river bottom. The post-parasitic stage can be considered the most critical stage in the life cycle of the FPM; during this phase, juvenile FPMs depend on suitable habitat (temperature, a stable well-oxygenated substrate with high sediment quality). If the above conditions do not meet the requirements, or there is not a sufficient amount of quality feed available, young FPM die. This interrupts the entire reproductive cycle, even if the other stages (glochidia maturation, host fish invasion, and metamorphosis) are successful. Juvenile FPM excyst from the host tissue when they reach a size of 0.3–0.5 mm (Hastie & Young 2003). Using its foot, the juvenile mussels bury themselves in the substrate, where they remain until they are large enough to anchor themselves to stream beds. According to experience in the Czech Republic (Hruška 1999), juveniles remain in the bottom for about five to ten years, at which point they emerge from the river bed to occupy a stable position on the surface. However, even older cohorts can be submerged in the bottom to a significant extent (**Fig. 3**). Sexual maturity varies with population longevity (Meyers & Millemann 1977, Young & Williams 1984). In our conditions, the age range is between 15 and 20 years. Once FPM are sexually mature, their increments do not reach previous values because most of their energy is used to produce eggs or sperm. The life expectancy of FPM in the Czech Republic in cold mountain locations usually exceeds 100 years. The oldest individuals, aged over 110 years, have been found in the Blanice River (Simon et al. 2015).

Fig. 3. Well-anchored adolescent FPM with large and clearly visible growth.

Chapter 3: Key factors endangering freshwater pearl mussel populations

Author: Ing. Kateřina Rambousková, Ing. Věra Kladivová, Mgr. Jan Švanyga, Mgr. Ondřej Simon
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- Eutrophication caused by land use around streams and in wider river basins
- Drainage and other amelioration on agricultural and forest land and associated erosion in river basins
- Watercourse regulation
- Inappropriate agricultural and forestry management and land use change
- Mass tourism
- Inappropriate fishing management
- Inadequate wastewater management
- Introduction of non-native species into river basins

3.1 Eutrophication in wider river basins

In addition to atmospheric deposition (nitrogen), the main reason for the increasing nutrient content (nitrogen, phosphorus, etc.) in watercourses (i.e. the process of eutrophication) is mainly agricultural production. At higher altitudes, for example in the Šumava Mountains (Bohemian Forest), in the second half of the 20th century, mineral fertilizers were widely used to increase agricultural production. Compared to organic fertilizers, they are not able to sufficiently bind to the soil complex (Vrba & Huleš 2007). Thus, they are leached into surface water and groundwater (Záhora et al. 2011), which leads to an artificial increase in the nutrient amount in the environment, the result of which is an increase in the mortality rate in all age categories of FPM (Araujo & Ramos 2001). This does not apply only to the Czech Republic. In eleven of fifteen European countries, eutrophication is considered one of the main causes of the decline in the numbers of FPM (Bauer & Wächtler 2001, Geist 2010). However, the process of eutrophication can be aggravated by inappropriate application of organic fertilizers, inadequate wastewater management, or intensive pastoral farming in river basins. **Fig. 4** shows the relationship between nitrate-nitrogen concentration in the water (the source is mainly fertilizers or excrements) and the amount of agricultural land in river basins with FPM.

One of the most fundamental eutrophication factors that limit the natural reproduction of FPM in the wild is the high content of dissolved nutrients (especially nitrogenous substances) in the aquatic environment amounting to milligrams and micrograms in one litre of water. The limit level of nitrate content influencing the successful rearing of juvenile stages (young individuals) is 2.5 mg/l NO3- (Absolon & Hruška 1999). Many authors of European conservation programmes deal with different environmental values, such as 1.7 mg/l N-NO3- (that is 7,5 NO3-), which was too high for succesful reproduction (Larsen 2005) or 0.125 mg/l NO3-, what was a median concentration in rivers with succesful reproduction (Moorkes et al. 2007). Another important value is the content of phosphates or total phosphorus. The Czech Action Plan (CAP FPM) (Absolon & Hruška 1999) suggests the limit value of 35 µg/l of total phosphorus. Other limit indicators can be found in **Annex 1** or see Simon et al. 2015 Supplements.

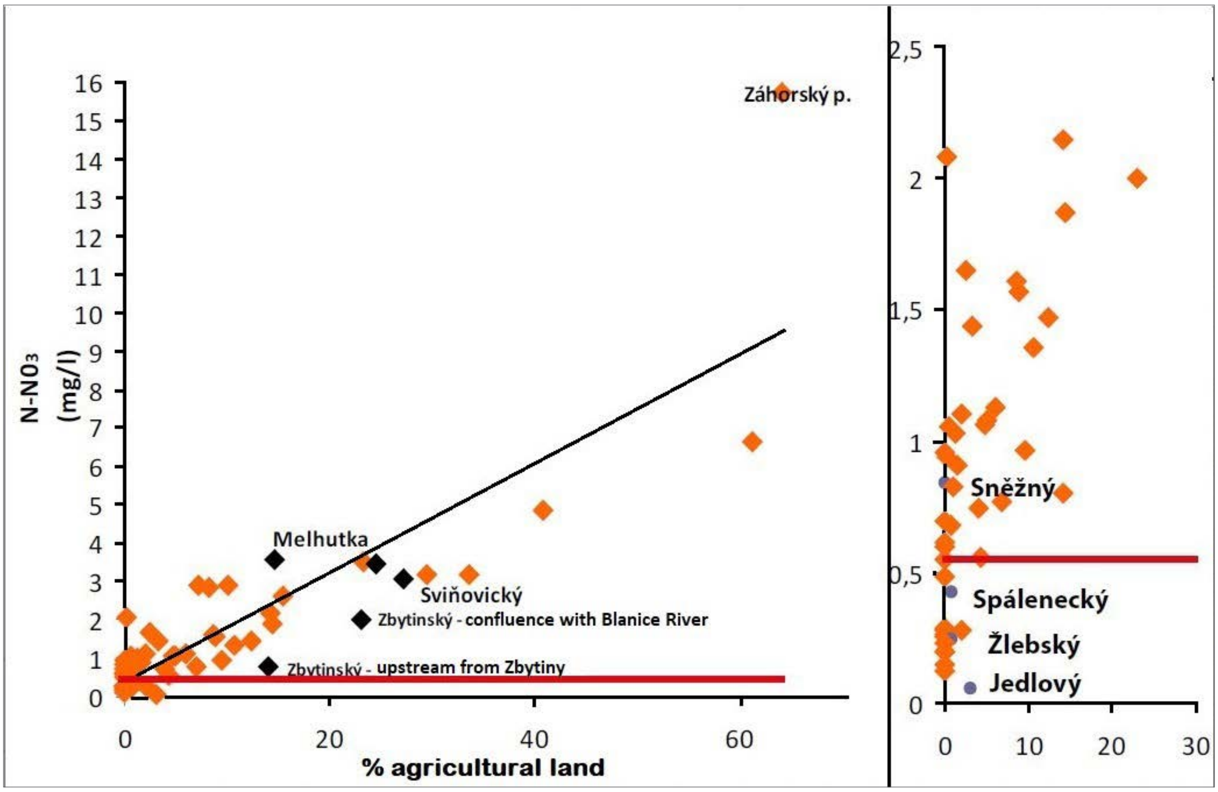

Fig. 4. Relationship between the nitrate-nitrogen amount in the water originating from agricultural production and the amount of agricultural land in a river basin (N = 63). The red line shows the limit value of nitrate-nitrogen (0.57 N-NO3 mg/l) for the FPM occurrence. The right part of the graph shows river basins (purple circles) where revitalization measures were applied (extensification of pastures, conversion of arable land into permanent grassland, etc.) and thus, the amount of nitrate was reduced below the limit value in most of them.

3.2 Drainage and other amelioration on agricultural and forest land and the associated erosion in river basins

The original state of the landscape has changed in many ways. One of the most significant activities of man in the historically long-term economic use of land was the draining of originally waterlogged habitats and wetlands. Wherever groundwater has naturally reached the surface in the form of fens or helocrene springs (spring wetlands that do not produce wells or dips directly from the springs), humans have tended to lower or significantly control groundwater levels for better land access and economic management (**Fig. 5**). According to the Ministry of Agriculture of the Czech Republic, 1.1 million ha (25 %) of agricultural land was drained in the Czech Republic in the past (MZE ČR 2012). As a result, wetlands are nowadays one of the most endangered habitats in the country. Therefore, in addition to national legislation, they are also protected by international treaties, such as the Ramsar Convention of 1971 (ratified in the Czech Republic in 1990).

In the Šumava Mountains in basins of the Blanice River and the Zlatý potok stream, the helocrene springs and water trickles are the main source of detritus, a fine decomposed organic material that serves as a food for juvenile (young), subadult (adolescent), and adult FPM. Detritus is transported to the FPM colonies by tiny tributaries and streams, which is only possible in places of good interconnection of the entire basin. Management of meadows and arable land over the last 50 years has led

Fig. 5. Drained pasture in Vyšný (an extinct settlement near the village of Křišťanov).

the groundwater deeper into pipe systems, which has generally cooled it, and prevented the development of wetland vegetation on the surface. For this reason, among other things, the food sources for the FPM have been eliminated.

In the case of forest land, the construction of drainage ditches caused severe erosion processes, which are nowadays a regular source of large amounts of fine-grained sediments in many streams. Fine sediments subsequently cover river beds where FPM occur, clog interstitial (inner) spaces in the river bottom inhabited by juveniles, and, moreover, make adult colonies unstable.

Inconsiderate management of valuable wetland habitats affects not only the FPM but also the reproduction ability of *Salmo trutta m. fario* (the only host fish of its larval stages) and many other aquatic and wetland organisms.

3.3 Watercourse regulation

The need to control the flow of water in streams and rivers extends deep into history. Whether it was for floating timber, the construction of water mills and iron mills, or the later production of electricity, shipping, or flood protection, man has always interfered with the natural development of rivers and streams with the aim of controlling water. The morphological nature of streams is very important for FPM, though. From the hydrodynamic point of view, it mainly depends on the structure of the bottom substrate, the mosaic of different types of environment (microhabitats), and current ratios. Of the physical parameters, the temperature curve or the saturation of the hyporheal by oxygen is essential.

Construction of reservoirs, weirs, and locks had far-reaching consequences, especially for the dynamics of watercourses and their migration permeability. The construction of the Vrané reservoir on the Vltava River and the Labe River lock in Střekov (both were completed in 1936) definitively stopped the migration of *Salmo salar*, the main host fish of the FPM.

The reservoir in the town of Husinec (built in 1939) interrupted the river continuum on the upper Blanice River and made it impossible for the populations of FPM and Salmo trutta m. fario to interact below the dam. The construction of the Lipno reservoir and the entire Vltava Cascade significantly changed the current conditions and dynamics throughout the whole Vltava River, and thus the fish assemblage structure. All these modifications resulted in a fundamental change in conditions for both the FPM and its host, *Salmo trutta m. fario* (**Fig. 6**).

Some aspects of watercourse regulation may bring partial positive effects for the conservation and promotion of FPM. For example, the construction of mill races has created a number of semi-natural habitats with a suitable substrate and controlled hydrological conditions, suitable for both adult FPM and host fish. The Šebelů fish pond in the town of Husinec on the Blanice River, where a residual population of FPM lives, or the mill race at Fork's mill on the Zlatý potok stream in the village of Kralovice, used as a temporary refuge for rescue transfers, still serve as examples.

Mill races stopped juvenile and adult FPM from being transferred downstream by the rivers, which increased the numbers of the bivalve molluscs at these sites. The mill races themselves, however, were unable to provide good reproduction conditions. In the past, efforts were made to establish so-called mill race reserves for FPM reproduction support; however, this effect has not been achieved, neither in the Czech Republic nor abroad. This also applies to the Horažďovice mill race on the Otava River, where FPM from the river were brought down to protect them from poachers and reportedly "multiplied" unprecedentedly. However, these reports have not been substantiated. This was also confirmed by V. Dyk, who had been dealing with FPM since the 1940s, and also recommended the establishment of these mill race reservations at the beginning, along with stocking of *Phoxinus phoxinus* (minnow) and *Cottus gobio* (bullhead) as alleged glochidia hosts, which also proved to be wrong (Dyk 1947, 1992).

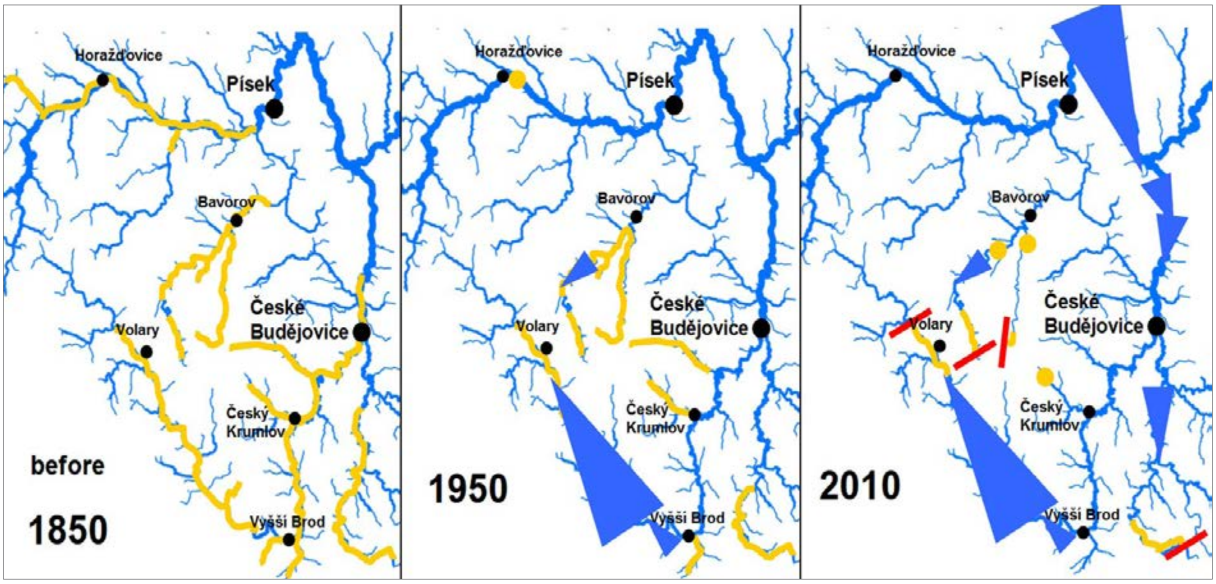

Fig. 6. Gradual extinction of the FPM (yellow sections of water courses) in southern Bohemia due to water pollution and the construction of water works on streams (blue triangles). The upper elevation limit of species distribution (caused mainly by the low water temperature of higher situated sections) is indicated by red lines. After SIMON ET AL. (2012).

Another suitable environment for FPM are semi-natural side streams, the so-called rearing and reproductive features (RRF), built in the framework of the rescue programme of FPM just for the purpose of effective control of the environment for the earliest stages of bred FPM. All these technical works represent some sort of rescue elements in extreme water conditions in the main streams and support the natural survival of the species at a particular location, while ensuring regular appropriate care.

However, in the long run, these are unstable parts of the river network. At present, a number of historically functional sanctuaries have ceased to exist due to their gradual aging or, in contrast, their technical renewal, for example to use water for small hydroelectric power plants.

3.4 Inappropriate agricultural and forestry management and land use change

The main areas of today's occurrence of FPM (for more details see **Chapter 4**), namely the foothills of the Šumava Mountains and the Gratzen Mountains (Novohradské hory), or Trojmezí in the Aš region, were rather intensively farmed before World War II. The nature of pre-war land use, however, was diametrically opposed to that of the post-war landscape mosaic, as well as the intensity and structure of agricultural and forestry production. Dramatic changes in land use are illustrated by numerous studies and publications, such as Bílý et al. (2008) or Skokanová et al. (2012). Originally a fine mosaic of tiny patches of meadows and pastures was transformed into larger complexes after the Sudeten expulsion; headlands were ploughed and many pastures were turned into intensively cultivated fields. Many areas of historical forest-free land have spontaneously overgrown through natural regeneration or have been specifically forested with *Picea* sp. (spruce). In most cases, forest growth was re-expanded into an afforestation of man-deforested river floodplains. However, the result of this process is a noticeable **cooling of the whole river basins**, where the temperature curve does not correspond to the limit for natural reproduction of FPM, that is temperatures above 15 °C in a continuous ten-day period (Absolon & Hruška 1999). The issue of the influence of low water temperature on FPM is discussed in **Appendix 2**.

The change in post-war use of forest-free land has led, among other things, to the gradual degradation of alluvial *Alopecurus* meadows (among others) to sedge fallows, which, as stated by Blažková & Hruška (1999) and Blažková (2010), leads to undesirable changes in the composition, quality, and availability of detritus from dead rhizosphere (roots) of these grasslands. FPM then lose food sources. **Chapters 5** and **6** deal with vegetation and detritus in detail.

Over the last two decades, the structure of agricultural production has been partially remedied, with a number of poorly cultivated fields turning back into pastures. However, another problem has arisen here, and this concerns **the incorporation of springs and small watercourses into cattle grazing areas**, where not only are biologically valuable habitats destroyed, but the FPM colonies are also directly threatened by turbidity and faeces. A correct example of small-scale fencing, which can easily prevent these problems, is shown in **Fig. 7**. Sumps, for example, can also be dangerous due to permanent housing of cattle in a close proximity to a water course with FPM, as well as cattle slurry on the meadows or the digestate and fugate from biogas plants.

Some hunting practices (inappropriate placement of salt licks, medical licks, feeding places, and rushes to springs and near streams) can also have a negative effect. The threat to important wetland communities is mainly due to the higher level of eutrophication and mechanical damage to the soil surface and vegetation caused by the concentration of game, or due to the introduction of ruderal and non-native plant seeds in the form of feed.

Fig. 7. Correct integration of the revitalized Sviňovická strouha stream into a pasture area with a full fencing of the surrounding zone.

In 2009, the Czech Environmental Inspectorate issued a decision to the Vojenské lesy a statky ČR (military forestry organisation) setting out the storage and post-production treatment of timber, the use of transport routes, and the restoration of drainage ditches in the National Natural Monument Blanice in the Boletice military area. The reason for this was up to ten times exceedance of the limit values for FPM for selected indicators of the water quality in the Blanice River and its tributaries (the flows corresponded to phenol-, phosphorus-, and ammonium-containing putrefactive waters). This was the result of inappropriate work procedures for damage recovery after hurricane Kyrill. By following the conditions of this decision, and consistent internal control activities, this forestry organization now achieves high standards in protecting the habitat of the FPM and the quality of runoff in the Special Area of Conservation (SAC) Boletice. This case pointed to the need for a careful approach to forest management, with an emphasis on the protection of springs and fine river networks, especially in waterlogged areas.

The current state of river basins in South Bohemia was documented by chemical screening of the area in the spring of 2016. Three main river basin groups were included: (A) human watershed areas with significant effects of farmland flushes, erosion, and drainage systems; (B) river basins with well-preserved oligotrophic (nutrient-poor) natural character; and (C) catchment areas where effective protection has brought back nutrient-poor levels. The effect of land use on water chemistry in FPM catchment basins is illustrated in **Appendix 3**. Based on the collected data, it is evident that in river basins in protected areas, where intensive farming stopped, water quality has already improved so that it again meets the very strict demands of FPM. Nitrate and phosphate concentrations have decreased, as well as conductivity (i.e. total ion concentration in the water); the concentration of dissolved calcium has also decreased. Although calcium is needed for FPM to build a shell, it can be used only in the form attached to organic particles they receive in their diet.

3.5 Mass tourism

Tourism has a growing trend worldwide and is no different in the Czech Republic. Thus, long-term sparsely populated areas often experience an unprecedented recovery, which goes hand in hand with the development of tourist infrastructure and increasing pressure on natural resources. From the point of view of the FPM protection, the Aš region is an example of this phenomenon. Neither the upper Malše River, nor the upper Blanice River are main tourist destinations. However, the situation is different at the Teplá Vltava River. This river flows through the core zone of the Šumava National

Park and is a popular destination for both canoeists and fishermen. For example, as Simon & Kladivová (2006) report in their study, in 2005 over 10,000 boats were recorded on the Teplá Vltava River in the Soumarský Most – Pěkná section, with a daily maximum of 400 and an hourly maximum of up to 120 vessels. This extreme load was then reflected in the state of the entire ecosystem of the river. In addition to uncontrolled movement of tourists in the first zone of the National Park and the accumulation of rubbish, there was a strong damage to stands of aquatic plants (macrophytes), which are the main source of detritus for FPM (Hruška 1996, Dort 2009). Boating has been regulated since 2009. The Šumava National Park administration sets the water level limit for boating (canoes, kayaks), the hourly and daily maximum of boats in the season, as well as a fees that also cover guides for groups of boats. This system works well and provides a good compromise between water tourism in the National Park and nature protection.

FPM can certainly be regarded as the so-called umbrella species of oligotrophic basins, from whose protection a whole range of other organisms benefit. FPM can also be regarded as a flagship species, whose protection can be presented to the public and popularly presented in the field of biology, ecology, and nature conservation. However, it is necessary to carefully and sensitively select the degree of disclosure of information about FPM localities; for the very effort to protect the species may ultimately turn against the species itself. This is evident from the case of the stream Lužní potok (on the German side of the Aš region), where, after allowing access to a specific location and installing an information panel, the entire local colony gradually disappeared as a result of illegal handling and collection.

3.6 Inappropriate fishing management

Another threat to viability of the FPM populations may be the lack of suitable hosts for the development of larval stages. In the Czech Republic, only the *Salmo trutta* m. *fario* is currently the host fish for glochidia (larval stages) of FPM. During the long joint development of both species, a strong link between the parasite and the host arose, whose specificity increases with the geographical proximity of both populations (Geist & Kuehn 2005). For example, the introduction of non-indigenous trout stocklines or the restocking of other non-native salmonids, such as *Oncorhynchus mykiss* (rainbow trout), can disrupt this fragile bond. Such introductions can disrupt the purity of the original trout gene pool and, indirectly, threaten the population of FPM due to different fish immune responses after glochidia attachment to gills. Studies in Germany (Altmüller & Dettmer 2006) and in Norway (Larsen 2005) have shown that the highest success rates (for parasitic invasion of trout) occur when the FPM and trout come from the same basin, i.e. trout from a local population. Luckily, the local trout stocklines in the Šumava Mountains are used in the Blanice River and in the Teplá Vltava River.

A low number of host fish on the site may also be a major problem. For example, the Teplá Vltava River suffers from a shortage of host fish. This reduces the success of glochidia attachment, and thus the overall success of natural reproduction of FPM. Therefore, it is advisable to support the natural reproduction of the original trout as much as possible.

The construction of reservoirs and ponds in a catchment area can also strongly influence fish communities. This is the case above the Lipno reservoir, where in the spring *Aspius aspius* (asp) and *Squalius cephalus* (chub) are thrusting upstream in the Vltava River and causing competitive and predatory displacement of local trout into smaller tributaries. The result is the absence of trout in the main bed at the time of glochidia release (Hladík et al. 2015). Likewise, the construction of ponds in catchment areas with FPM adversely affects the whole system, whether it is clogging the bottom with fine mud or allowing *Cyprinus carpio* (pond carp) and *Perca fluviatilis* (perch) to escape into the trout zone. In addition, the construction of flow-through ponds creates new impenetrable barriers on watercourses, which present an obstacle to both natural fish migration and drift (passive current drift) of adult FPM. Furthermore, all flow-through reservoirs capture organic detritus, formed in the springs area, which is a necessary food for FPM to optimize their juvenile stages.

Fishing management and interactions of salmonid populations with local populations of FPM are not further elaborated in this methodology; the Ministry of the Environment of the Czech Republic is preparing a special methodology for this important topic.

3.7 Unsatisfactory wastewater management

As human society has developed economically, the number of extraneous substances entering the aquatic environment has gradually increased. Toxic water pollution caused by the growth of manufacturing and industry in the 19th century gradually destroyed most historical FPM localities in the Czech lands (Simon et al. 2015). The population in the lower flows of the Otava and Vltava Rivers, the original centre of the species distribution area (Dyk 1992), disappeared completely. In the second half of the 20th century, this phenomenon spread to the so far little affected areas of these streams.

The main pollutant groups include industrial wastewater (with direct toxic effect), toxic metals, and specifically acting substances such as pesticides or medicines. Metals are present in the water in both the toxic and non-toxic forms, and their level of toxicity to fresh water molluscs is mainly due to pH (Buddensiek et al. 1993). Generally, juvenile FPM are more sensitive to toxic metal fractions than adults, and so there might be situations when a large adult population is able to reproduce but juvenile cohorts are missing.

An example is the stream Volarský potok, polluting the Teplá Vltava River with wastewater of high conductivity and episodically high values of ammonium ions. In 2014, water sampling and bioindication demonstrated the link between an increase in ammonium ion concentration above 1 mg/l NH_4^+ and 100 % death rate of juvenile FPM (Černá et al. 2017). Similarly, the stream Zbytinský potok used to be a source of pollution for the Blanice River. A significant positive change in this case was building of a sewage treatment plant with purification and retention ponds in the village of Zbytiny (Simon et al. 2010b). At the present time, good growth and high survival rates have been documented in juvenile FPM in the lower part of the Zbytinský potok stream (see **Chapter 5.7**).

3.8 Introduction of non-native species into the river basin

The introduction of non-native species (animals, plants, microorganisms, etc.) into FPM catchments always poses significant risks not only to the FPM but also to the entire ecosystem.

An example of a dangerous introduction of an invasive animal species can be the confirmed occurrence (in 2017) of *Pacifastacus leniusculus* (North American signal crayfish) in the upper Malše River. Recent research has shown that this invasive crayfish, which is a predator of juvenile and subadult stages of FPM, has caused a decrease in its populations (Schmidt & Vandré 2012, Sousa et al. 2015). *Pacifastacus leniusculus* is also a very strong competitor for our indigenous crayfish species and a carrier of the dangerous crayfish plague (Alderman 1997), which can subsequently destroy entire populations of native crayfish species such as *Astacus astacus* (European crayfish) and *Austropotamobius torrentium* (stone crayfish). Thus, a seemingly insignificant introduction of a single animal may result in substantial changes in the functioning of the entire ecosystem.

Chapter 4: State of Freshwater pearl mussel populations in the Czech Republic

Author: Mgr. Ondřej Simon

Freshwater pearl mussel (FPM) have been brought to the brink of extinction in the Czech environment; their former distribution has been reduced by more than 95 %. In the last known localities, only small remnants of the original million colonies are represented (Matasová et al. 2013, Simon et al. 2015). There are more causes of this massive decline in the Czech Republic and around the world, as described in more detail in **Chapter 3**. It is probable that most rivers with FPM were destroyed due to the discharge of industrial wastewater from urban areas. In terms of smaller streams, both large reduction in the number of individuals and the extinction were caused by changes in landscape management, water course straightening, land drainage, and generally neglected care of water purity by residents and local governments (Dyk 1947, 1992, Hruška 1991, Flasar 1992) – see **Figure 8**.

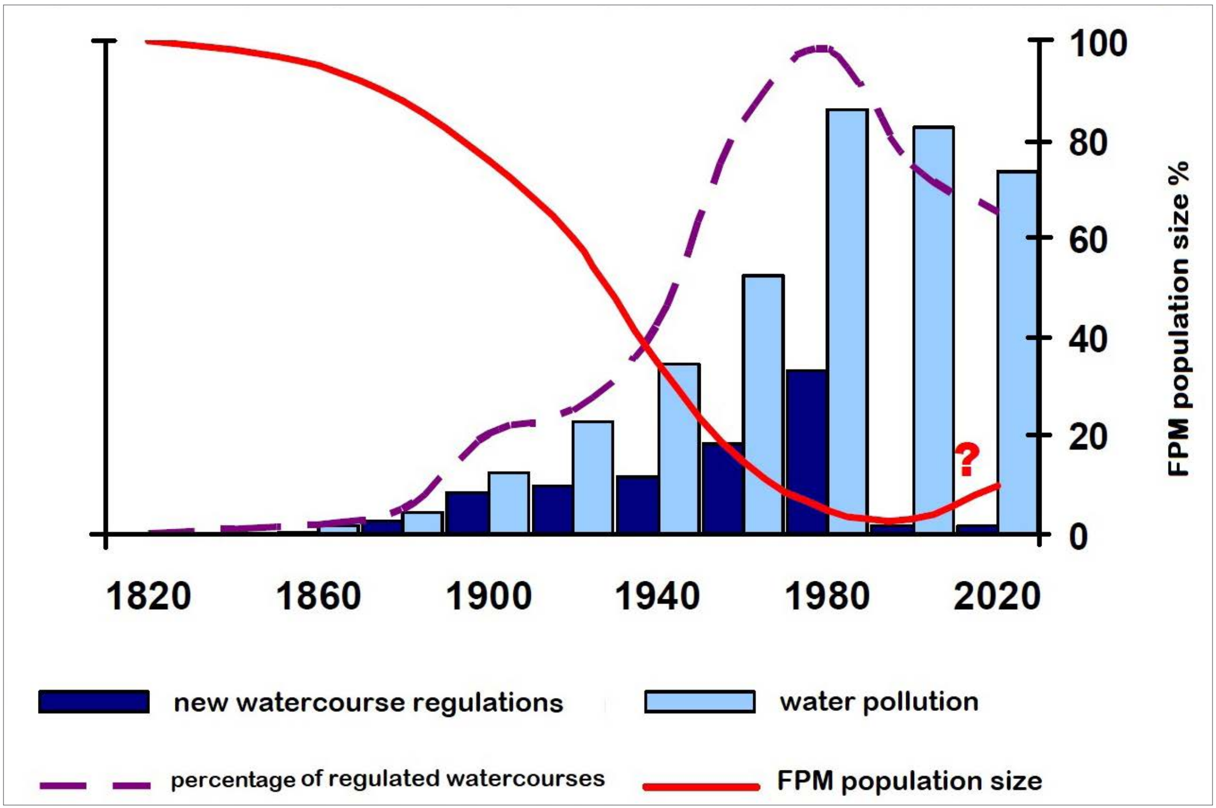

Fig. 8. Relationship between extinction of FPM in Czech lands over the last two hundred years and the level of water pollution and watercourse regulation. Although pollution often decreases rapidly, watercourses remain regulated (currently a large part of the river network in the Czech Republic is regulated in some way; a large part is also completely destroyed by pipelines). The percentage of regulated watercourses within the entire length of the river network includes primary source streams (expert estimate). Pollution is related to the maximum load of the river network in the second half of the 1980s, understood as 100 % (expert estimate). Improvements also include natural restoration and its effect on water purity.

At present, the last Czech populations of FPM occur in the following six river basins (mostly in border areas): Blanice River, Teplá Vltava River, Zlatý potok stream, and Malše River in southern Bohemia, Lužní potok stream in the Aš region, and Jankovský potok stream in the Bohemian-Moravian Highlands (**Fig. 9**). FPM live here because these streams are currently the only and last ones within our territory with satisfactory water chemistry, although other parameters for these streams do not provide optimal conditions (unsatisfactory temperature conditions of the aquatic environment, riverbed drying, floods, ice phenomena, erosion, insufficient stability of the bottom, etc.). The reason for this occurrence is not the fact that FPM are a typical species living in foothill and mountain rivers. The current residual populations of FPM do not live in an environment that corresponds to their historical distribution based on optimal conditions. Due to the reshaped Central European landscape, these remaining oligotrophic streams in sparsely populated foothills are currently the only possible refuges where this critically endangered species is still able to survive.

Three genetically distinct types of FPM have been found in the Czech Republic, as reported by Simon et al. (2015). Each of these populations is named after the area of its main occurrence (**Fig. 46**); the Saale population (Saale River basin in the Aš region), the Blanice River population (brooks and river basins of the upper Vltava and Blanice Rivers), and the Malše River population (the Malše River and the upper Vltava River before it reaches the Lipno Reservoir). Local types of FPM (also referred to as conservation units) are adapted to live in specific rivers and **must not be mixed during breeding or conservation operations**.

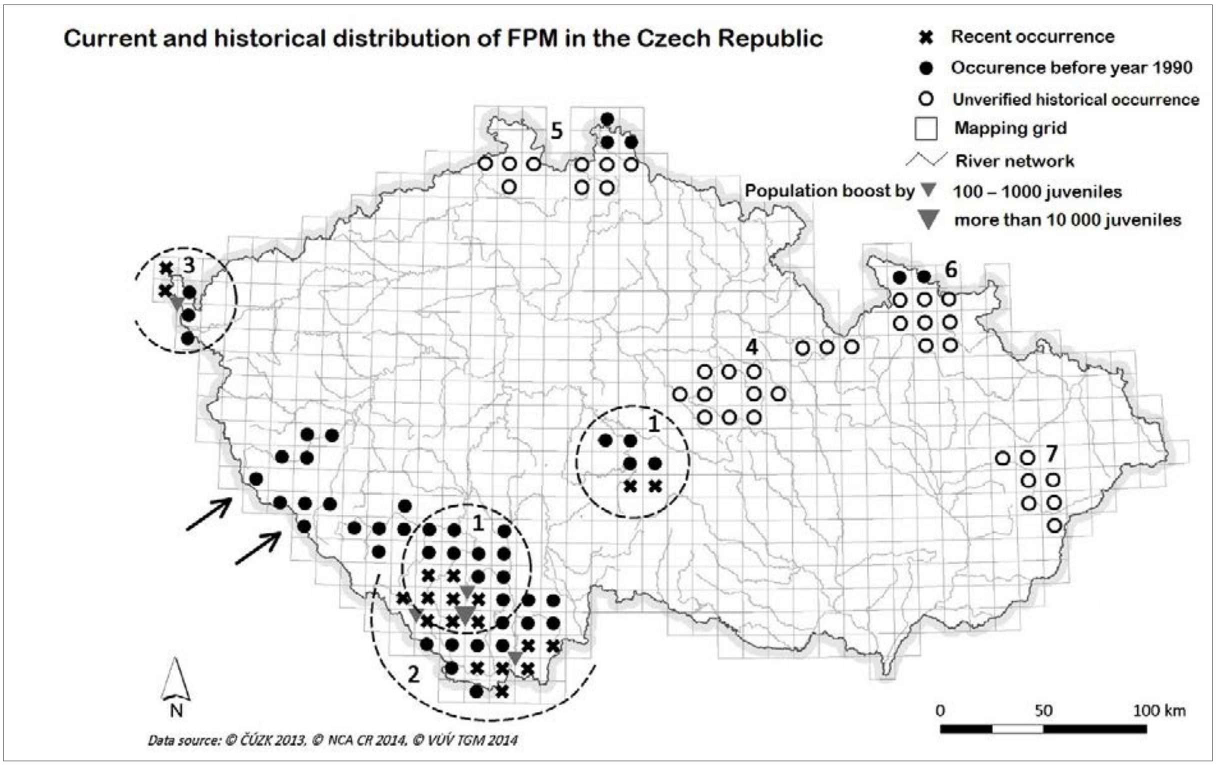

Fig. 9. Current and historical distribution of FPM in the Czech Republic, after Simon et al. (2015). Extinct populations and uncertain historically reported FPM localities: (4) rivers Orlice, Doubrava, Chrudimka; (5) rivers Lužická Nisa, Ploučnice; (6) Kladská Nisa River; (7) Bečva River. Recent occurrence of FPM: (1) Blanice River, Zlatý potok stream, Teplá Vltava River, Jankovský potok stream; (2) Malše River and a part of Teplá Vltava River; (3) Lužní potok stream, Bystřina stream (Saale River basin). Arrows on SW border of the Czech Republic point to the sterams Švarcava, Kouba, and Kamenný potok (Danube River basin), which flow from the Czech Republic into Bavaria, Germany; FPM still occur in these streams.

Just as people in the last century lost the opportunity to drink water from a stream or river, the FPM has lost much of its natural habitat. It is the overall revitalization of streams and the following changes in the water regime of river basins suitable for FPM, that enable it to survive. In addition, future generations of people will be able to enjoy their presence. From a pragmatic point of view, all these measures helping to preserve the current distribution of FPM are also aimed at saving our declining sources of drinking water.

Chapter 5: Methodology of procedures to ensure food supply for freshwater pearl mussel using special restorations

Authors: Mgr. Ondřej Simon, Ing. Michaela Černá, Jaroslav Hruška, Bohumil Dort, Ing. Kateřina Rambousková

Reviewer: Ing. Karel Douda, Ph.D., Ing. Ondřej Spisar, Ph.D., Jaroslav Hruška

During the first stages of the Czech Action Plan (CAP FPM), it was possible to prove that young juveniles and in some cases even older individuals are often limited by lack of food at remaining Czech sites (Hruška 1995). However, it was not an absolute lack of particles in water or hyporheal; the problem was the indigestibility of some fraction of these particles (clay particles, ferric precipitates). The most common problem was the absence of some nutrients, especially calcium.

Improvement of the environmental conditions necessary for natural reproduction and growth of pearl mussel then proceeded in two basic directions. Primarily, efforts were made to restore the entire catchment area above their site so that, even without any special management, nutritious detritus was produced (connection to the helocrenic spring areas (see **Annex 5**), primary river net restoration, and eliminating erosion and pollution). Catchment oriented conservation is evidenced by the name of the first stage of the CAP FPM – *Conservation of oligotrophic waters by the method of active protection of the habitat and the population of freshwater pearl mussel in the Czech Republic*. However, due to the organizational and time-consuming work on the restoration of the entire catchment, special side arms (Rearing and Reproductive Features – RRF) and composting meadow management designed for pearl mussel were established to reach the above goal (according to the so-called Czech method aimed at supporting reared juveniles).

In this chapter, we want to describe in more detail the issue of pearl mussel food and evaluate the various procedures to supply river net with detritus, which should lead to a local improvement in pearl mussel diet. Furthermore, some laboratory experiments will be described aimed at identifying the quality of detritus and possibilities for improvement it for rearing purposes.

5.1 Freshwater pearl mussel diet

The issue concerning the nutritional requirements of FPM is very extensive and still little researched. So far, the possibilities of improving food supply in the scale of microhabitats and small river basins have only been examined and tested in the Czech Republic.

5.1.1 Juvenile and adult diets

The scientific literature agrees that the diet of FPM adults consists of small particles (smaller than ca. 40 µm) filtered from the water column. Adults from the oligotrophic stream sections filter food particles through an expanded siphon (**Fig. 10**); young individuals can actively filter areas below the river bed or they feed by wiping food with their muscular legs and cilia – so called pedal feeding. The food in typical habitats is usually detritus with particles smaller than 40 µm, not living algal cells. This distinguishes the FPM from most other large freshwater bivalve molluscs, whose diet mainly consists of living cells of unicellular algae (Bauer & Wächtler 2001). FPM juveniles have an adaptation to filtrate particles twenty-times smaller than 40 µm (Lavictoire et al. 2018; Schartum et al. 2017).

The scientific literature hardly deals with the issue of food of juvenile individuals in natural in situ conditions. Rearing experience shows good utilization of organic detritus of suitable composition, such as that originated from helocrenic springs or from draining water from alluvial *Alopecurus* meadows (Hruška 1995, 1999, 2000b) or in combination with other fine particles such as some algal cultures or microbial suspensions (Eybe et al. 2013). **Chapter 6** deals in more detail with the Czech experience of food sources during a rearing process (including the wider context). A lot of researchers carry out pearl mussel rearing using semi-natural systems, such as side arms or semi-natural raceways connected to an external source of the river, helocrenic springs or lake water (Hruška 1999, Vandré et al. 2001, Preston et al. 2007, Lavictoire et al. 2016). However, in this case we do not know exactly what type of food the juveniles use.

5.1.2 Rearing and Reproductive Features (RRFs) and their influence on pearl mussel food supply

Although we still know little about the nutritional requirements of freshwater pearl mussel, as early as the 1990s it was shown experimentally that certain management practices on streams or stream beds and their surroundings improve juvenile growth downstream of these modified sites (Hruška 1991, 1992b, 1995). Auxiliary side arms (RRF) were proposed, which optimize conditions for juveniles in a small section of a newly established stream bed, especially with regard to food supply

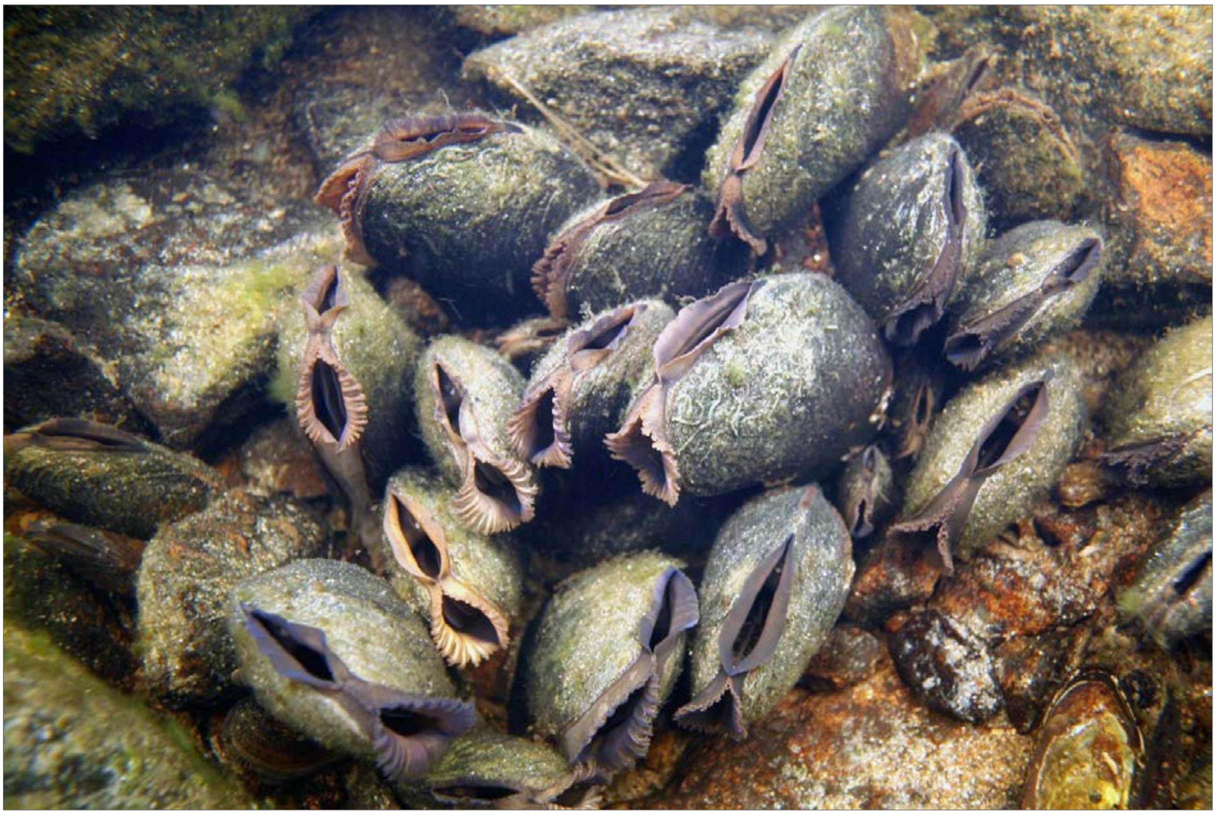

Fig. 10. A colony of FPM with adult and subadult individuals from the Aursunda River in central Norway. Wide-open receiving syphons with corrugated edges facilitate passive filtration, even at slow flow rates.

(which can be limiting for juveniles in Czech conditions) and hydraulic-morphological conditions. Young individuals are released into RRF. Here, they first find very good conditions for their development in the hyporheal and later they migrate independently to the main river stream.

The RRFs were developed and tested first at a small scale on the Blanice River (see **Figure 13** – view of the first restored RRF small-scale model). Subsequently, they were implemented in the Spálenecký potok stream in the Blanice River basin (Spálenecký RRF – SRRF, **Fig. 14, 15** and **18**), the middle course of the Lužní potok stream near the town of Aš (LRRF, **Fig. 17**), and the upper course of the Zlatý potok stream (ZRRF – based in natural floodplain soil, **Fig. 16**). Similar RRFs are being designed for the Malše River. Feeding of juveniles of 0+ and 1+ cohorts with detritus from helocrenic spring sites, RRF implementation and food stream construction are some of the basic procedures (Hruška 1992b, 2000a) that led the CAP FPM to the successful rearing of the first subadult cohort under natural conditions (Absolon & Hruška 1999, AOPK ČR 2013a, Simon et al. 2015). The individual RRFs are described in more detail in **Annex 4**, and their construction in **Chapter 10**.

Given the above-mentioned facts, it is necessary to consider the restoration of the function of springs and the whole river net in the catchment with pearl mussel as a basic method of special restoration (return to a natural state). In contrast, laborious special meadow management (**Fig. 16**) and the construction of other RRFs only represent a complementary temporary conservation method of management for natural communities of oligotrophic river basins with freshwater pearl mussel habitat, which is necessary only until the restoration of all the catchment is finished.

Fig. 11. A colony of FPM from the Vltava River basin in a section with faster flow and higher concentration of particles (adults only).

5.2 Food detritus characteristics

It is very difficult to define detritus. It is a very complex material based on fragile flakes of microorganisms and precipitated colloidal substances in combination with the remains of plant tissue from leaves or roots (usually up to a particle size of 1 mm; in hydrobiological literature so-called FPOM – fine particulate organic matter). Another important component is formed from solid faecal pellets of invertebrates (sand shrimps, mayfly larvae etc.) of various sizes and durability. Its other components are mainly clay and fine sand mineral particles. This complex structure is overgrown with bacteria in the form of a thin biofilm, algae cells, and a wide variety of unicellular organisms (especially "protozoa" and "fungi"). Furthermore, depending on the size of the sieve used in straining the detritus, there is a large amount of meiofauna (invertebrate organisms below 500 µm in size), such as Ostracods or Harpacticoida, and many minor stages of benthic organisms (*Chironomidae* – midges, Turbellaria, etc.). Detritus is, therefore, more conceivable as a complicated three-dimensional ecosystem with rich fauna and flora in micro-world scales rather than a homogeneous suspension of dust dispersed in water. The composition of detritus and its formation is shown graphically in **Figures 19** and **20**. A detailed chemical and biological composition of helocrene from the Blanice River basin used for juvenile feeding, including spring typology is available in **Annex 5** or in Tichá et al. (2020). Comprehensive overviews of the chemical composition of detritus for larger and smaller streams and rivers

Fig. 12. Traces of wiping of a thin layer of detritus from the surface of the rearing container with one-year-old juvenile – pedal feeding (Petri dish, diameter 20 mm, one individual during a bioindication experiment).

Fig. 13. The Blanice River – the first model of an RRF. Small food streams fertilized with compost (photo from early spring 2016 just after compost application) bring nutritionally rich detritus to the main stream at the place of colonies of young FPM individuals reared in the 1990s (Blanice River in the background).

Fig. 14. Composting site in Spálenecký potok stream RRF – SRRF. Right to left – special compost aged 1, 2, and 3 years. The compost is only applied after three years.

are also provided by Zimmermann-Timm (2002) and Wotton (2007). Hruška (1995) and Tichá et al. (2012, 2020) deal in their qualitative and quantitative study with a specific case of detritus coming from the Šumava floodplains and springs used in the CAP FPM (AOPK ČR 2013a).

Detritus is primarily caused by the decay, enrichment, and reprocessing of plant litter in the aquatic environment under oxic conditions. Fine flakes of detritus, unlike more stable leaves or wood, are easily washed off by flow from the spring area (**Fig. 20**). Their movement is interrupted by repeated settling and resuspendig as the flow rate increases, or as a result of the action of organisms such as *Arvicola amphibious* (European water vole), see for example Allan & Castillo (2007), Newbold et al. (2005), and others. Detritus is a very mobile component of organic matter and is rapidly transported downstream. Its more durable components disintegrate within 90 days (Wotton 2007). It can be transported very quickly, even at low water levels. At higher flow or stormflow, detritus transport spikes on a logarithmic scale (Wallace et al. 1997, 2007). The average distance, reached by a detritus particle was measured specifically in the Blanice watershed (Slavík et al. 2016).

Consequently, due to the structure of detritus, it is necessary to explain in more detail the way the FPM is adapted to obtaining food whilst growing; their body organs change (Lavictoire et al. 2018; Schartum et al. 2017), and thus different age stages of FPM use different forms of food (Hruška 1999, Bauer & Wächtler 2001). FPM stages up to 2 mm in size feed by filtering food particles by swirling cilia on the mantle; they can also use pedal feeding with cilia to wipe the surface (**Fig. 12**) of river bed particles and to drive the particles into their open shell. Its cilia filter out edible material, and the unwanted components are expelled like pseudopellets. Above 2 mm shell size, mantle-like papillae develop (similar to *Unio* and othe mussel papillae, see pictures in **Annex 9**), allowing them to more effectively take river bed sediments, but active food intake still prevails (Hruška 1999). Only much later, with a

Fig. 15. Aerial view of SRRF in the Blanice River floodplain. In the foreground: composting place. Photo: Petr Jan Juračka, taken from a drone.

Fig. 16. A stream with a stripe cut along it that is fertilized with compost in winter (ZRRF) (and Jaroslav Hruška).

Fig. 17. During the vegetation period the stream is completely lost in the grass (LRRF).

shell size between 35 and 40 mm, a fine receiving apparatus is formed around the receiving syphon, which is already used to passive feed on food drifting with the current (**Fig. 10**). That is why it is so important that an adequate food environment for the individual developmental stages is available. While the stages after excystement from the host up to a shell length of 35 to 40 mm need the flow-through interstitial of the river bed, larger individuals need a solid and clean gravel river bed, allowing firm anchoring and filtration of free-flowing water bringing food from distant sources.

5.3 Natural sources of detritus

Natural sources of food detritus (organic matter) may vary in the vast area populated by the freshwater pearl mussel. For good growth of individuals, stream sections below lakes (Preston et al. 2007, Ostrovsky & Popov 2011, Lavictoire et al. 2016) offer plankton decomposition as one possibility of detritus formation. In the hyporheal below the river bed, biofilm on the grains of the substrate may be a source of detritus (Pasco et al. 2015). In the Czech conditions of oligotrophic mountain streams, verified sources of detritus are submerged macrophytes (in the case of Teplá Vltava River, **Fig. 21**), helocrenic permanent springs, and rhizosphere of mesotrophic *Alopecurus* meadows (especially on the Blanice River, Lužní potok stream, Malše River, and Zlatý potok stream; Hruška 1995, 2000a, AOPK ČR 2013a; **Fig. 22**, see also **Chapter 9** for details). The sources of detritus are various, and consequently, detritus composition is various as well (Hruška 1995, Zimmermann-Timm 2002, Wotton 2007, Tichá et al. 2012) – for more details see, **Annex 5**.

Fig. 18. A view of stream cross-profile with overhanging banks from the frog's-eye view (SRRF).

In connection with different natural sources of detritus, it is also necessary to mention how different eutrophication work. Mineral substances dissolved in water – nitrates (NO_3^-), ammonium ions (NH_4^+), or phosphates (PO_4^-; the main component of reactive phosphorus) – are in principle always undesirable, sometimes directly toxic and damaging to the habitat. On the other hand, FPM need both nitrogen and phosphorus in a particulate organic form. Similarly, the above also applies to calcium. Calcium in dissolved forms is unusable for pearl mussel and its increasing concentrations lead to gradual destruction of favourable habitat conditions, although calcium itself is not directly toxic to pearl mussels. Although FPM are classified as calciphobic bivalve molluscs (see the limits of calcium according to the CAP FPM, AOPK ČR 2013a), they need a relatively high proportion of digestible organically bound calcium for the construction of a massive shell, especially in the juvenile stage of development. Dyk's experiments show that FPM can survive in the long term even in hard water with high calcium concentrations (Dyk 1947). However, the higher proportion of divalent cations (calcium, magnesium) in water leads to rapid coagulation of drifted detritus, which makes up the diet of FPM. For this reason, freshwater pearl mussels prefer streams with very low concentrations of dissolved calcium and magnesium, where they can feed on small suspended particles filtered from flowing water (**Annex 6**, Hruška 1995).

Thus, although there should be almost no dissolved nutrients and calcium in the water, their content in detritus in particulate form is necessary for the life of FPM. It is submerged macrophyte vegetation, spring ecosystems, or roots of certain meadow grasses that mediate this transfer from dissolved particles to particulate organic form.

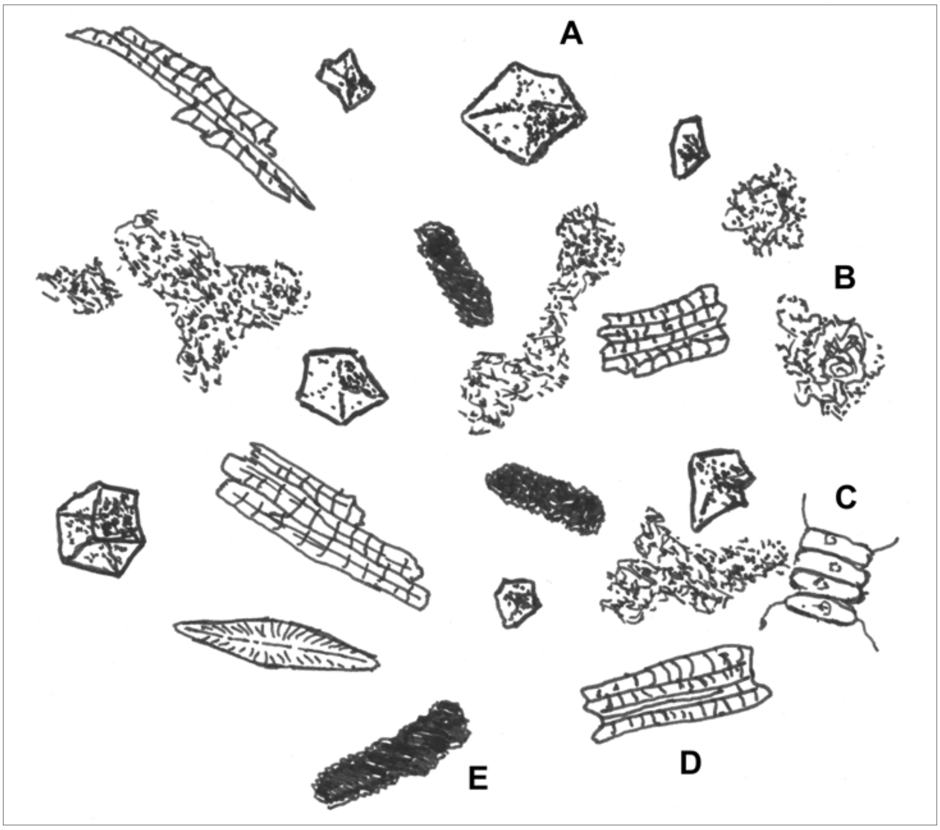

Fig. 19. Demonstration of microcosm of detritus magnified about a thousand times. A – clay and mineral particles, B – flakes of microorganisms and precipitated colloids, C –algae cells, D – plant tissues residuas, E – faecal pellets. For reasons of clarity, the individual particles of detritus are not to scale. Drawing: Michal Bílý.

5.4 Quality of food detritus for freshwater pearl mussel

Given the demands of FPM, either an insufficient amount of detritus or its unsatisfactory chemical composition may be limiting. In the Czech Republic, insufficient amounts of detritus were usually not recorded in the summer season. Water courses with FPM sites are usually fast-flowing and, even at low flow rates, the amount of suspended organic solids is sufficient due to intensive resuspension; their concentrations do not fall below cca 2 mg/l at the monitored sites (Tichá et al. 2020). Data concerning total suspended solids in water are available for most FPM localities of the Czech Republic from some years on the NCA CR website (https://www.zachranneprogramy.cz/perlorodka-ricni/data-vuv-t-g-m-v-v-i/).

Particularly problematic is the unsatisfactory composition of detritus. The first problem is a significant proportion of completely indigestible inorganic substances, such as clay particles, precipitated iron, or small sand particles in detritus. Their amount is determined by chemical analysis (**Fig. 23, 24**) as so-called ash (residue after burning of the dried sample, AFDM analysis). If major erosion occurs in the basin, drifting detritus is very rich in mineral particles and is unusable for FPM. They can only feed on an organic component, depending on its nutritional value given by the proportion of carbon and other components (**Fig. 25**).

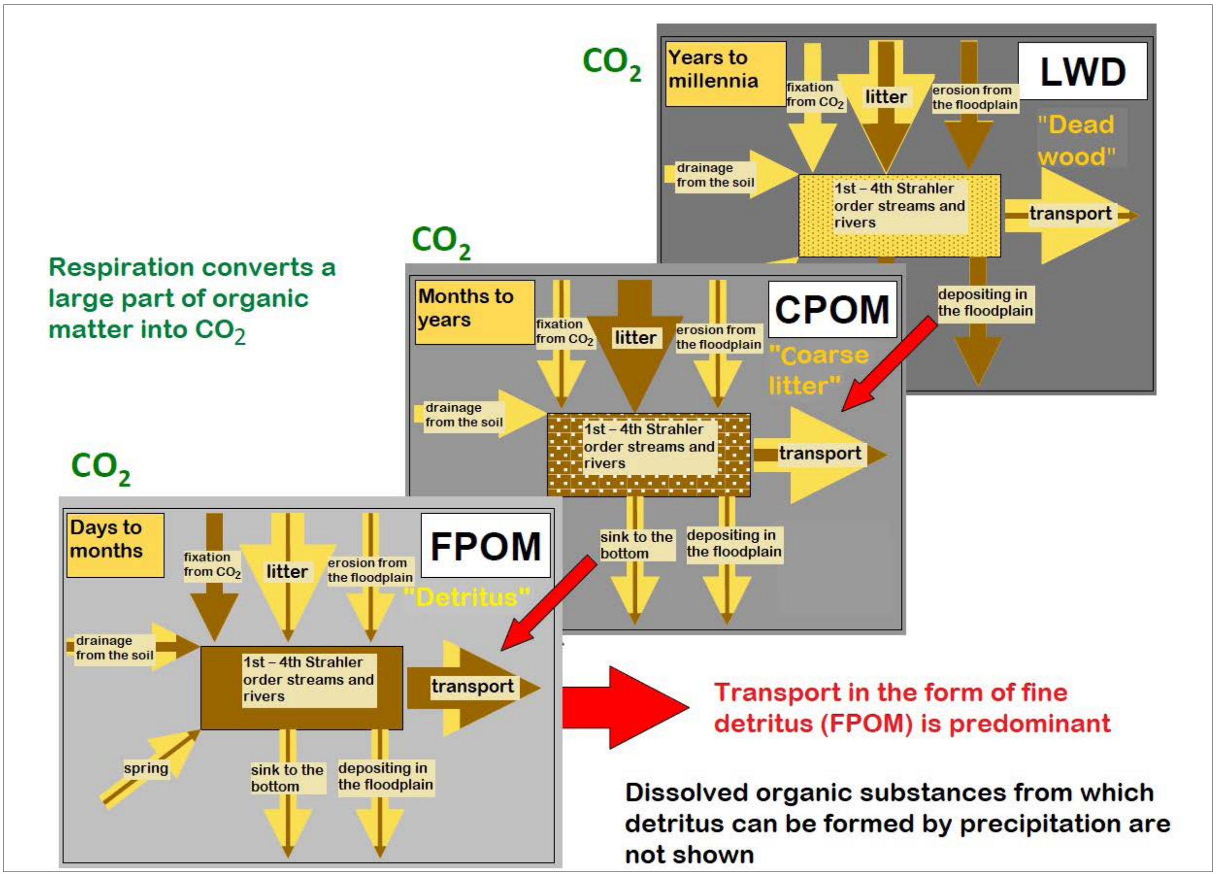

Fig. 20. Detritus formation using the example of streams and rivers (1st – 4th Strahler order) in the Blanice River basin, Šumava Mountains. The red arrows show the rate of shifting between size fractions and predominant downstream transport. LWD – large pieces of wood, CPOM – coarse litter (e.g. leaves, twigs, tree fruit), FPOM – fine detritus of size under 1 mm, coloured part of the arrow shows the relative importance of the given pathway transmission. Adapted from Simon et al. (2008).

In the case of iron, the influence of the ferric ochre (iron oxides) flushed out from stagnant wetlands into a stream is often very important. Bright orange to rusty ochre is mixed with favourable food detritus and degrades it. Juvenile FPM do not accept a mixture of particles where ferric ochre is present. With the help of cilia and mucus they form pseudopellets that are then ejected, together with high-quality detritus. This leads to starvation, weakening, with mucus and energy depletion. Therefore, stagnant wetlands and outflows from drained acid soils with high production of ferrous ochre in the water are very unfavourable in FPM habitats.

The calcium availability is also essential. The FPM only uses organic forms of calcium to create its massive calcareous shell (Hruška 1995). Calcium concentrations in detritus vary widely in both locality and size fractions (**Fig. 26**, all size fractions are visible). However, pearl mussels can only directly use some fractions smaller than 63 µm. Moreover, large particles and flakes may disintegrate into smaller components during transport from the helocrenic springs to colony sites in main river channel.

Another important property of detritus that is possible to measure chemically is the ratio of nitrogenous substances (mostly proteins) in comparison with the amount of carbon (mostly in indigestible organic particles) – the so-called C:N ratio. An example of how the C:N ratio changes in the longitudinal profile of the Vltava River is shown in **Figure 27**. Detritus with a C:N ratio of less than 14:1 is considered to be a suitable diet in laboratory experiments. However, research into this issue is only at the beginning.

Determining the individual components of detritus by chemical analysis is costly and slow. Therefore, in practice, the method of bioindication by juvenile pearl mussels is used to evaluate measures taken to improve the rate increase. The FPM thus directly show whether the detritus at a particular site contributes to faster growth (**Fig. 28**) or whether it is toxic to them. Bioindications are discussed in more detail in **Chapter 7.**

5.5 Measures to improve the supply of detritus flows

For the above-mentioned reasons, measures are primarily aimed at improving the content of organic calcium in detritus, improving the transport of detritus from production sites to sites where pearl mussels occur, as well as reducing the content of mineral components in detritus resulting from excessive erosion and siltation in river net. Both the temperature conditions and maintenance of high water quality are equally important for the proper supply of detritus streams, especially with regard to nutrient content, precipitated forms of iron, and conductivity.

The main measures used include:

- special composting management (improving the supply of calcium and nutrients and, at the same time, imitating historic management of alluvial meadows);
- restoration of primary river streams (transport and natural production of detritus from rhizosphere improvement);
- prevention of excessive erosion in the primary river network (reduction of mineral content in detritus);
- building of RRFs (complex improvement of detritus supply at a micro-scale);
- and overall river basin restoration (improving large-scale supply and preventing toxic pollution of detritus). A specific description of the function of the measures is contained in **Chapters 9** and **10**.

All the measures implemented so far have been applied in the upper, still unpolluted area of FPM occurence, where the species is currently approaching its survival limits. These are mainly due to the naturally low water temperature and low content of nutrient detritus in the aquatic environment. On the other hand, eutrophication and toxic pollution are limiting in the middle and lower part of the species distribution in the Czech Republic (nowadays polluted streams with predominantly extinct populations – Simon et al. 2015, **Fig. 6**). The nutritional value of detritus is usually not limiting here (Hruška & Bauer 1995).

5.6 Experimental bioindication comparison of natural, modified, and artificial food

Experiments comparing various natural detritus, modified detritus, and artificial food showed some interesting results. Various experiments based on bioindication carried out in the laboratory are described in detail in **Chapter 7.**

Natural detritus from the Vltava River Meander site (previously known for stable and high-quality detritus) was compared with other modified variants. Both blended detritus and detritus with added homogenate (blended filtered detritus) were tested. The homogenate was also tested separately, diluted in stream water. Further, the following were prepared: analogues of detritus (artificial foods) with different concentrations of calcium and nitrogen (**Fig. 29**), leachate from peat mountain meadow soil (**Fig. 30**, **31**), and mixtures thereof in various concentrations. In addition, a macroinvertebrate homogenate from the Studená Vltava River as a high-nutrient substitute for detritus was used.

Fig. 21. The source of detritus in mountain rivers, such as the Teplá Vltava, is the rich vegetation of submerged plants. *Callitriche* sp. (water starwort) on the surface, and *Myriophyllum alterniflorum* (alternate water-milfoil) underwater in the background.

Natural river detritus (Vltava River Meander site deposition detritus), modified by blending a portion of its volume to break up living cells and reduce particles, was assessed as the most viable type for individuals of 1+cohort. In **Figure 32** it is shown in yellow. Artificially prepared food (red bars) based on macroinvertebrate homogenates, local soil extract, composted plant matter etc. led to low growth or even death of all individuals (in the case of compost).

An important question was whether FPM would grow without being fed with detritus (**Fig. 33**). A 60-day experiment showed that they grew for some time at first (see also white bars in **Fig. 32**), comparable to the individuals fed with detritus from the Vltava River. Thirty days later, however, growth slowed significantly. In comparison with the ten-day increment shown with different types of detritus, the growth was smaller on average. However, the average mortality in control samples without feeding was significantly small (around 15 %) within the entire 60-day period.

Therefore, natural or slightly modified detritus based on detritus from the Teplá Vltava River above the Volarský potok tributary proved to be the most favourable for growth of Vltava pearl mussel juveniles. Blending changed the form from particulated to dissolved phase, which probably improved the availability of some substances or minerals. As for artificial detritus, the growth was apparently low due to an unbalanced substance ratio. Based on these results, high additions of calcium, nitrogenous substances, phosphorus compounds, or freely available proteins are not a good way to accelerate the growth of pearl mussels in Czech mountains populations. The results have not been published yet.

In general, we can say that natural food has already managed to bring semi-natural rearing to the fertility stage, which has not yet been published anywhere in the world. In contrast, artificial,

Fig. 22. Rhizosphere as a source of detritus. Grass root systems on mesotrophic meadows growing on permeable substrate are also a source of detritus (Spálenecký potok stream, SRRF).

Fig. 23. Dobrá Hydrobiological Station Laboratory, Šumava National Park.: pipetting of detritus for bioindication experiments – Jan Švanyga.

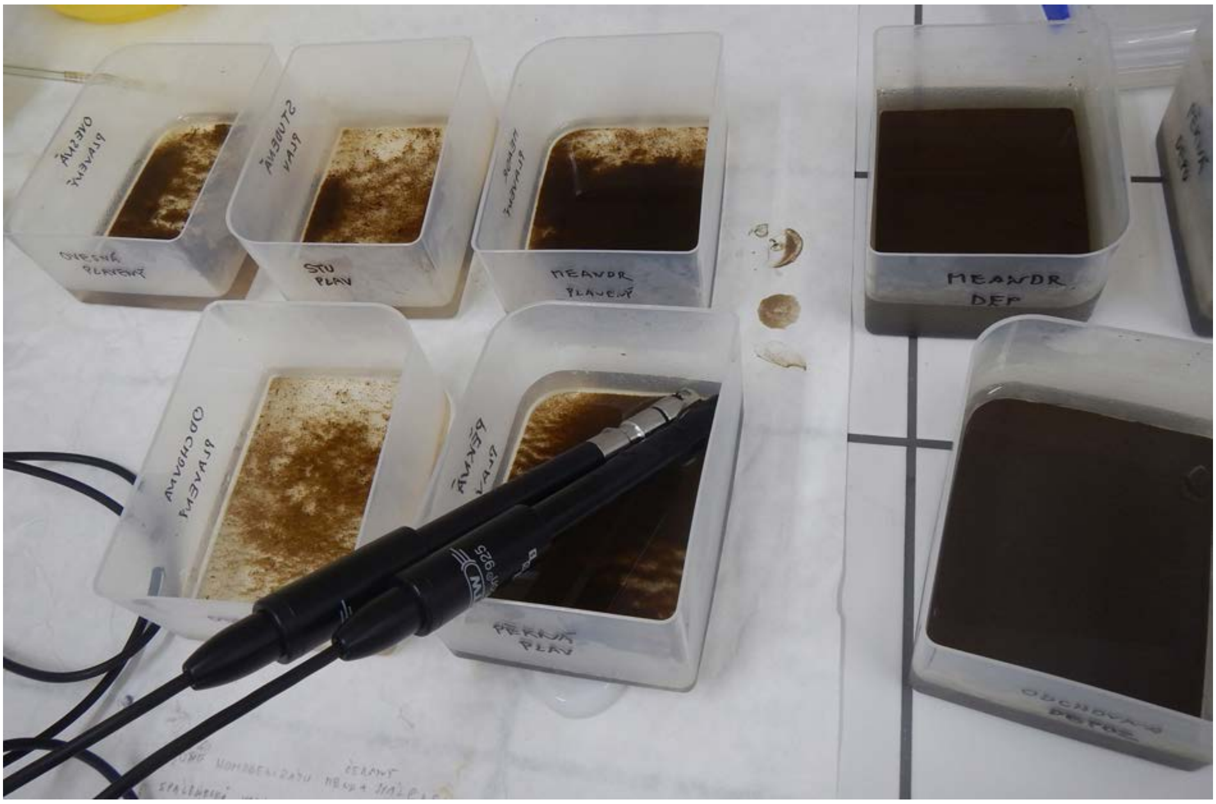

Fig. 24. Various types and colours of natural detritus used in the tests. Detritus water characteristics probe measurement.

nutrient-rich food can lead to uncertain results in the long term. The risk is, for example, the selection of juveniles predisposed for more eutrophic conditions, which after release will not survive in poor and cold locations. However, it is possible that, in contrast to other organisms, the FPM is resistant to described selection.

Therefore, we propose to carry out a long-term experiment that will allow comparison of natural and artificial food after 15 to 20 years. It is necessary to establish a sufficiently large experimental group fed in the first year with the nutrient rich artificial commercial mixture. Only if these individuals can establish themselves in the natural hyporheal, and if they are subsequently found in subadult or adult age on the surface of the river bed, will we be able to generally recommend artificial food in general. It is short-sighted from the perspective of a long-term conservation programme to use juvenile rapid growth and low mortality as an argument. Furthermore, in the Czech Republic it is not possible to apply widespread breeding practices, which are successful in short-lived pearl mussel populations living on the lower elevation range limit of the species. However, they could be beneficial, for example, on the course of the Malše River or the Blanice River under the Husinec reservoir, where middle-lived population survives in very small numbers – see **Figure 6**. This topic, therefore, deserve detailed elaboration in the future.

5.7 Evaluation of the impact of improvement measures on detritus quality

Within the CAP FPM activities, several evaluations of the empirically recognized positive impact of RRF on detritus were carried out (for more details on RRFs, see **Chapter 10**). Most of the tests focused on the bioindication assessment of various types of detritus after improvement measures, the experiments concerning the growth of juvenile FPM individuals, and specifically the determination of calcium content in detritus or plant litter.

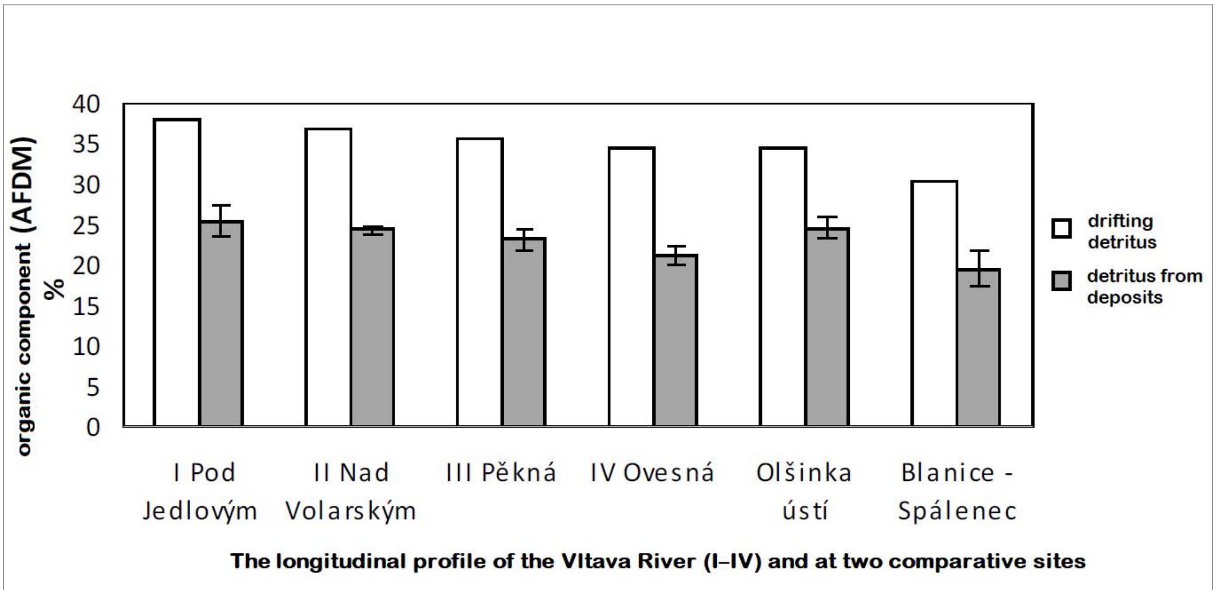

Fig. 25. Comparison of organic fraction in detritus (AFDM) in the longitudinal profile of the Vltava River (I–IV) and at two comparative sites. Detritus from deposits – three repeated samplings, drifting detritus – seven-day representative mixed sample from sedimentation trap DDP (see Chapter 7.3.2.2) installation in the river.

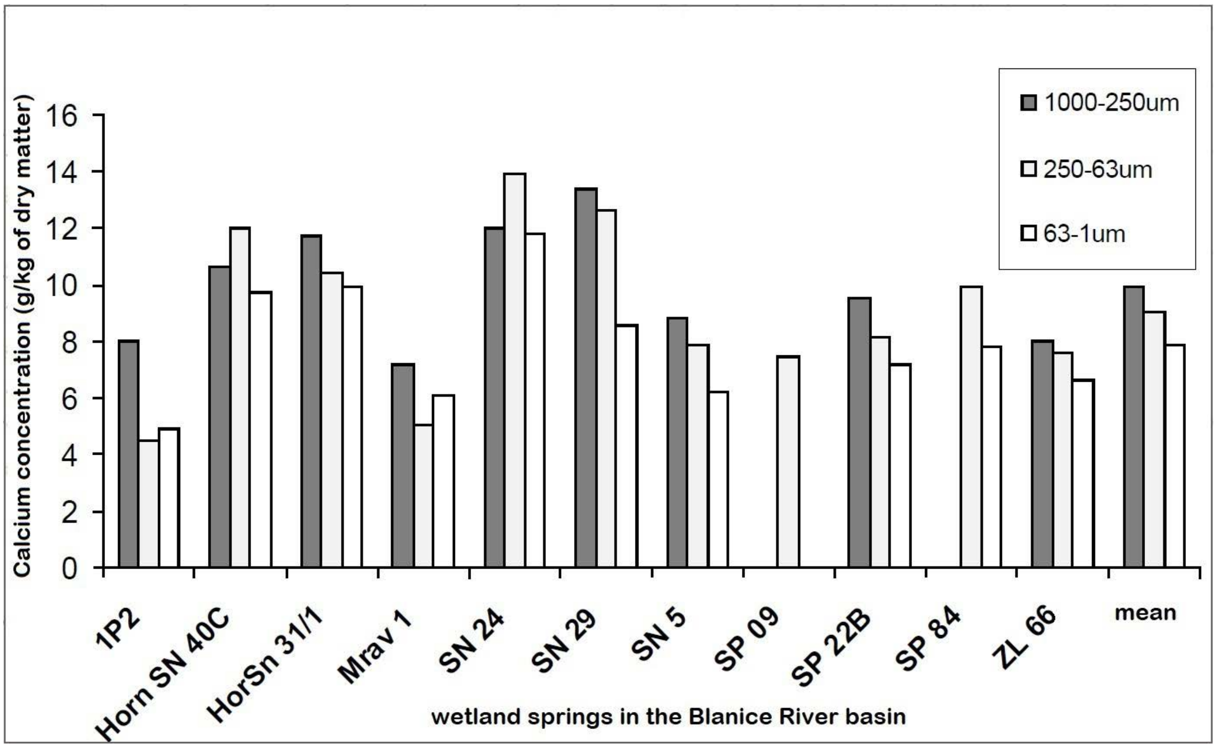

Fig. 26. Calcium concentration in detritus flakes of three size fractions (1–0.25 mm, 0.25–0.06 mm, and less than 0.06 mm – without vacuum filtration) of drifting detritus flowing from 11 wetland springs in the Blanice River basin (for details see Tichá et al. 2020).

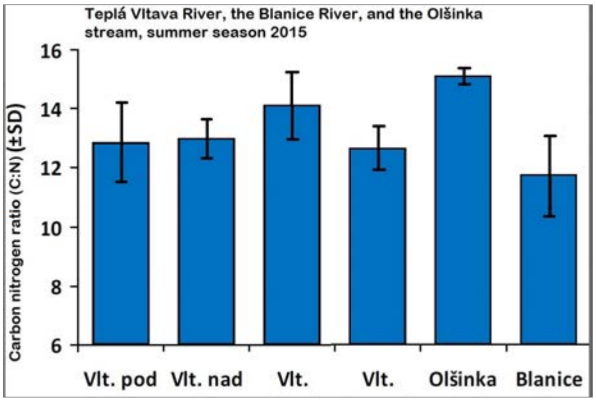

Fig. 27. Carbon nitrogen ratio (C:N) as a measure of the content of digestible substances in deposited detritus in the Teplá Vltava River, the Blanice River (most favourable average value), and the dystrophic Olšinka stream (least favourable value), summer season 2015.

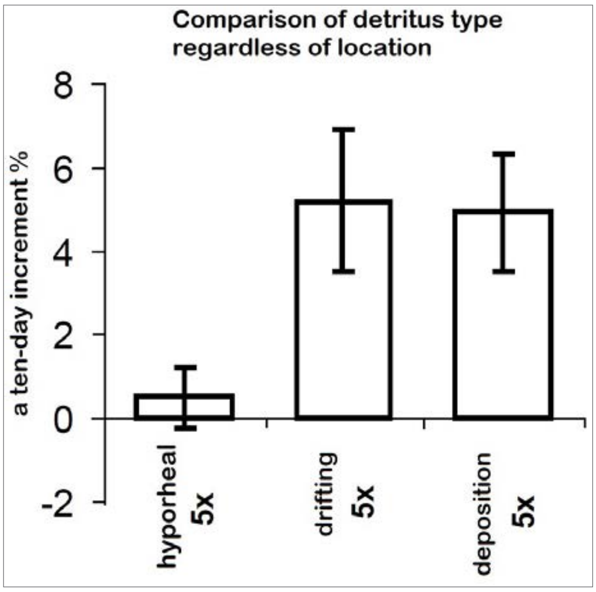

Fig. 28. Comparison of percentage increase of juvenile FPM in a laboratory test of detritus from five sites on the Teplá Vltava River taken from hyporheal (significantly smaller increment of juveniles), from deposition, and from drifting detritus suspended in water column (similar juvenile increments). Drifting detritus was taken from the water column with a DDP sampler (similar device like in Fig. 85, for details see Patent CZ 303836).

5.7.1 Results of detritus quality improvement measures – available data

A lack of organic calcium is a fundamental problem for detritus quality, which is currently receiving a lot of attention when implementing various measures. The first data on calcium content in detritus and other organic materials was presented in pioneering works from the late 1980s (Hruška 1991). Calcium, phosphorus and nitrogen content in detritus from a set of pristine helocrenic springs together with quantitative measurements from Blanice River catchment was published recently by Tichá et al. (2020). Subsequent long-term botanical studies have confirmed major differences in calcium content in above-ground and underground biomass, which results in alimentary detritus in floodplain conditions. Botanical studies compared degraded sedge stands and species-rich habitats with *Alopecurus pratensis* (meadow foxtail) or *Poa* sp. (meadow-grass) restored by appropriate management (Hruška 1995, Blažková & Hruška 1999, Blažková 2010 – also see **Chapter 9** on meadow management).

The effect of forest liming was also evaluated in the Cikánský potok basin, in the Boubín massif (Hruška 2000a). The influence of massive areal application of lime was not proven at first. Significant improvement in the growth of FPM occurred only in the following years when calcium was incorporated into plant tissues, but this effect was not permanent. See **Annex 6** for more information on forestry management.

A comprehensive evaluation of the calcium content of detritus from springs with different management was carried out during the implementation of special restoration studies for the Blanice River and Zlatý potok stream and the Malše River basin in the Czech Republic and Austria (Dort & Hruška 2008, Dort 2009, 2012). Recently, data from deposited and drifting river detritus were also obtained from the Vltava River in Šumava National Park. Detritus from sections where strict protection was introduced in the NP in the 1990s, as compared to river basins affected by eutrophication (Černá et al. 2017). However, data on the influence of different methods of calcium uptake into the environment have not been published in detail.

Initial data on improved growth of FPM in rills with modified vegetation, calcium composting management, and modified artificial porous subsoil originate from the beginning of the 1990s, when the RRF system was developed (Hruška 1999). Current data on the direct influence on water chemistry are not available (see **Annex 4** for exceptions). The effect of the measure can be indirecty deduced only from bioindication tests.

In order to correctly interpret the results of bioindications, it is always necessary to simultaneously test a sufficient number of Buddensiek plates or Hruška sand cages at comparative sites to eliminate the effects of the year or part of the season, and to combine field and laboratory methods to eliminate the effects of temperature (see **Chapter 7**). However, only a small number of datasets meet these criteria. Below are some examples of evaluating measures to improve food supply through bioindicators.

5.7.2 Comparison of RRF and main flow using bioindication of five juvenile age groups

The most abundant data for assessing the effectiveness of the implemented RRF compared to the main river flow is from SRRF and the main stream of the Blanice River at the Spálenec RRF centre. The most detailed data available are from 2015, where five age groups of FPM were tested simultaneously in the field (in situ) and in the laboratory (ex situ) is described below. In **Figure 34** it is apparent that SRRF increments reach a maximum of about 60 % per season, compared to the RRF centre, where it is about 80 % except for the 2+ cohort (120 %). Youngest 0+ cohort juveniles at RRF centre died except for one individual. SRRF increments are therefore sufficient from the time of the juveniles' excystement from the fish, compared to the RRF centre, where the first year pearl mussels do not survive at all (which corresponds to long-term results from previous years and long-time absence of reproduction (Simon et al. 2015)). As for the 1+ year used for bioindication, average increments are slightly better in the RRF centre. The fastest growth in the 2+ age category and then a decrease in the percentage increase has been a well-known fact for a long time (Hruška 1999). However, repeatedly confirmed deaths of 0+ individuals and poor growth of 1+ individuals in the main water course of the Blanice River at the Spálenec RRF centre are of interest (see **Chapter 5.1.1**). This was confirmed even in the extremely warm year of 2015.

Future research should obtain more extensive data on conditions in the hyporheal bioindications (Bílý et al. 2020; Černá et al. 2017), as there are no such dramatic deaths in the 0+ cohort in Spálenec RRF centre.

5.7.3 Comparison of RRF with other sites in the river basins

SRRF and ZRRF were evaluated in 2015, together with a wide range of habitats in both river basins, using the standard test of a juvenile cohort 1+ (**Fig. 35** – grow rate and mortality). Both the sites of small streams and sites of the main stream of the Blanice River were included. Very low RRF mortality values were found from a comparison of mortality in other sites in the surrounding FPM river basins

Fig. 29. Preparation of artificial analogues of detritus in the laboratory. On the far left a filtration device, natural water for dilution in a beaker, at the bottom GF/C filters with a fixed fraction after filtration of the homogenate and a stainless steel bar mixer.

Fig. 30. Preparation of leachate from peat soil. Conductometer on the right.

Fig. 31. Leachate from peat soil.

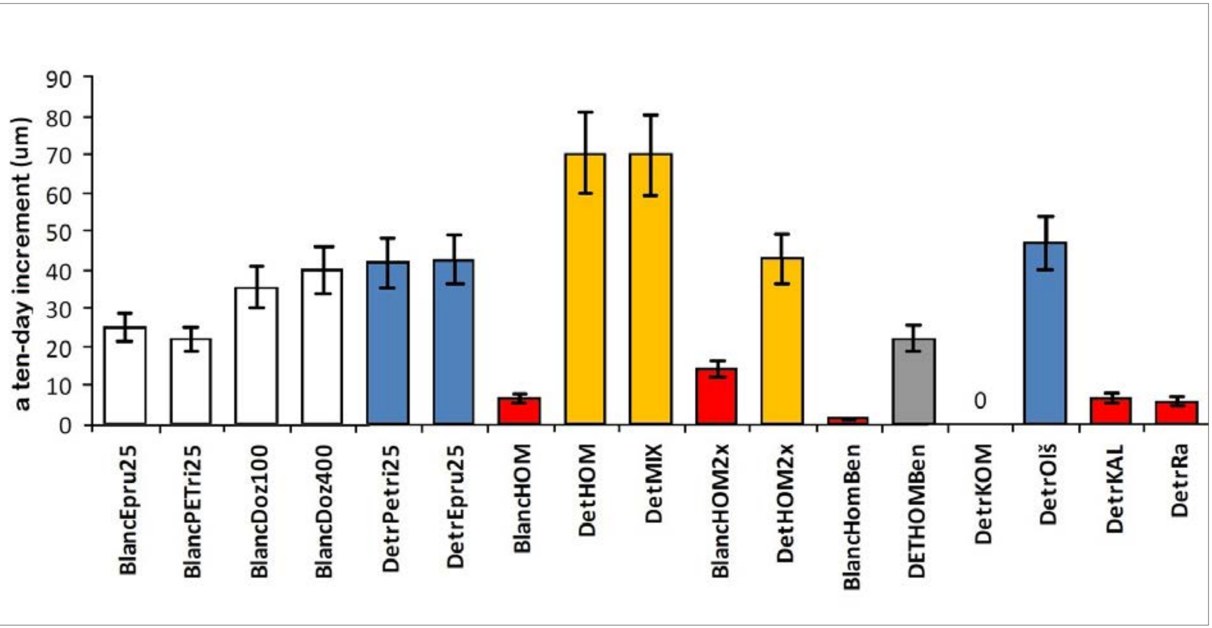

Fig. 32. Ten-day grow rate of juvenile FPM in a detritus-free environment (white), fed with river detritus (blue), fed with partially blended river detritus (yellow), fed with artificially enriched river detritus (grey), and artificial food (red). The artificially prepared food based on compost (DetrKOM) killed all individuals in the experiment.

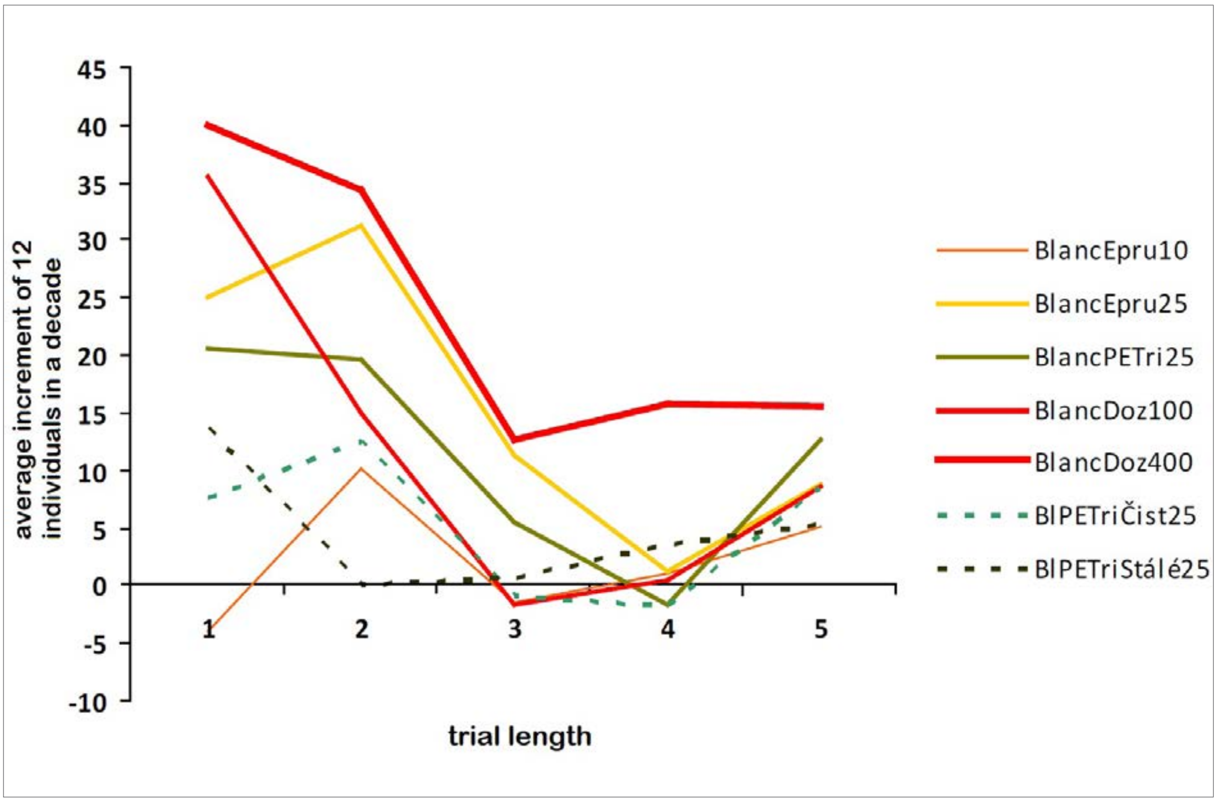

Fig. 33. Ten-day grow rate of 1+FPM cohort in a detritus-free water during in-situ indication. Control rearing of FPM in natural water from Spálenecký potok stream not fed with detritus for 60 days, measured after every 10 days. The number at the end of the series code indicates the volume of the container in ml, the hatched line shows the control with filtered natural water from Spálenecký potok stream (orange – test tubes, green – Petri dishes, red plastic food jars).

(**Fig. 35**). The mortality rate corresponds to other sites of small streams with low water temperature (highlighted by blue oval). The increment is low on both RRFs when compared to warmer main Blanice River sites (for more details, see **Annex 7**). The chart in **Figure 35** also presents other observed values from the same year and error bars to show the complexity of the issue and, at the same time, great variability of bioindication results (**Fig. 34** or **Annex 7**, **8**).

To eliminate the influence of temperature on assessing whether the growth of FPM in the RRF is improved due to the supply of high-quality detritus, it is necessary to carry out a constant-temperature test (ex situ) (the method is described in **Chapter 7**). However, less data is available for the 2015 test example. Therefore, SRRF detritus can be compared with a set of samples from Blanice River and the Tetřívčí potok stream basin. In this context, SRRF detritus used by FPM leads to an above-average grow rate when the effect of low temperature was eliminated (see **Annex 8**).

According to the results presented by Švanyga et al. (2013b), SRRF differed only slightly from the Blanice River main stream in the relatively cold year of 2012. To sum up, as a relatively well-functioning RRF, SRRF allows the growth of the youngest stages of pearl mussels, as opposed to the main Blanice River. Growth is slow but mortality is low. LRRF is discussed in more detail in **Annex 4**.

Another effect of the RRF, which has not been evaluated in detail, is the creation of a refuge where small FPM are protected from destruction during floods that shift the river bed in larger streams. **Figure 36** shows the SRRF site shortly after a major flood in 2006, which did not harm the local pearl mussels.

More detail on the evaluation of all three RRF are in **Annex 4**. However, it should be noted that, in particular from 2004–2012, we do not have enough data to monitor the functioning of most RRF. Much more work is needed to continue the detailed measurements from the time where these features were set up.

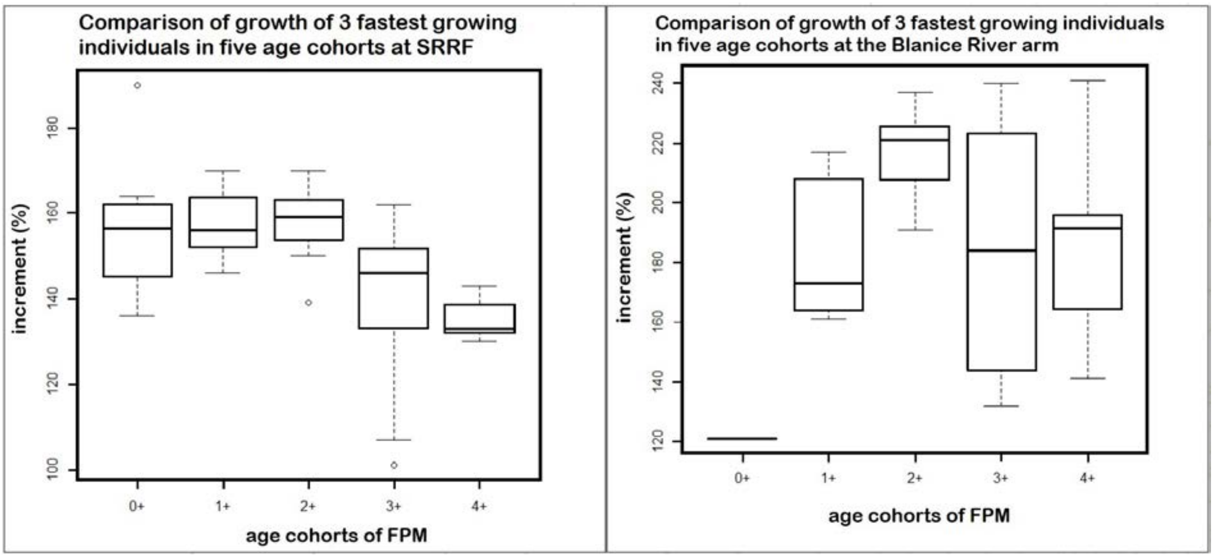

Fig. 34. Comparison of growth of five age cohorts of FPM (new individuals: 0+, up to individuals in the fourth growth period: 4+) in SRRF (left) and Blanice River arm (right). The percentage increase for one three-month summer season in 2015 is shown.

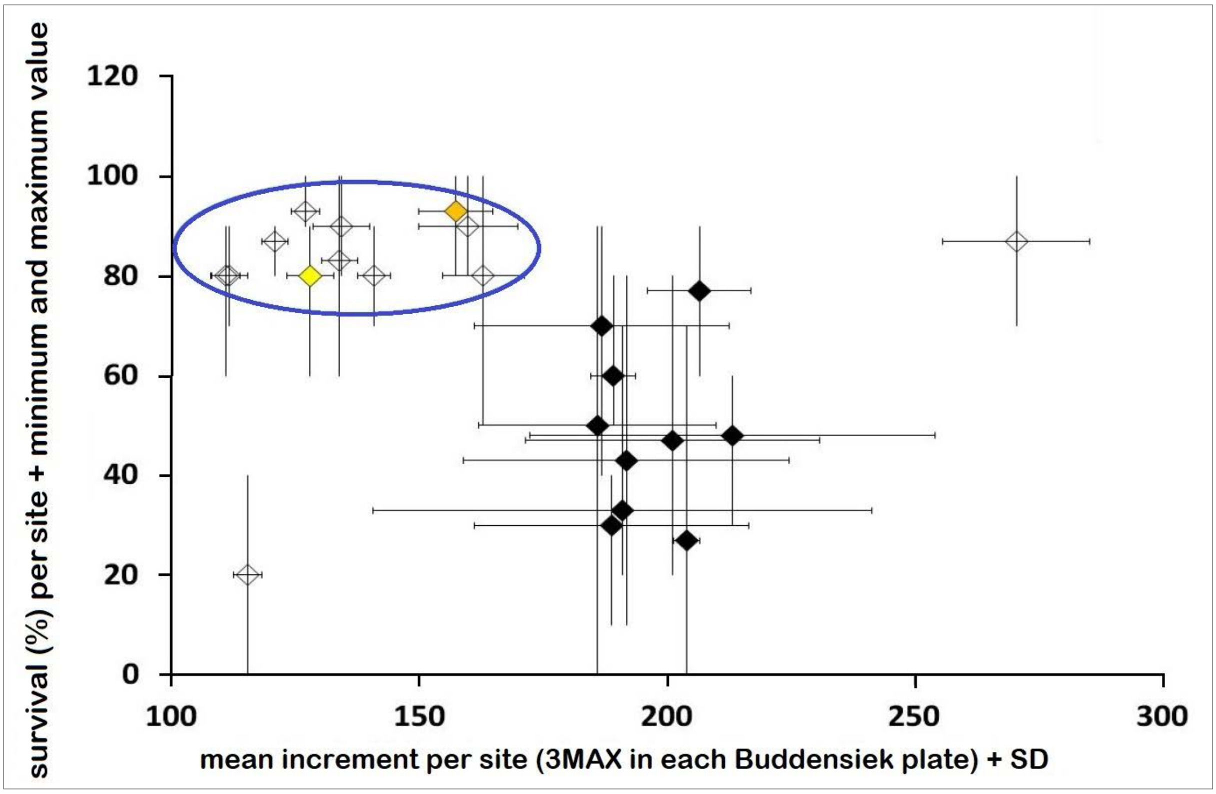

Fig. 35. Mean mortality of 1+ FPM expressed as percentage of survival and a percentage grow rate in RRFs on the Blanice River and Zlatý potok stream (SRRF – dark yellow, ZRRF – light yellow) in 2015 compared to other sites in basins (Blanice River main stream – black marks, other small streams – empty signs). The blue oval in the chart indicates small cooler streams in both river basins. For comparison, the very cold Hornosněženský potok stream (a reference site) is bottom left, and the mouth of Zbytinský potok stream (an eutrophicated site) is top right. SD shown by error bars, N= 14 individuals per site in two Buddensiek plates, for 3MAX characteristic see (Bílý et al. 2018).

Fig. 36. RRF on Spálenecký potok stream in the Blanice River floodplain after 10-year floods. There was no mechanical damage of rills or sediment silting in the SRRF.

5.8 Summary of knowledge about FPM food requirements

A great deal of knowledge has been gathered over the past thirty years from the study of FPM food, which has opened up many possibilities for its improvement. We can empirically verify, using bioindications, whether or not individual restoration structures or managements have an improving effect on the growth of juvenile individuals. This allows us to verify in practice the effectiveness of partial management interventions. If we know which spring areas, tributaries, food streams, or other sources produce nutritious detritus, we can adjust the procedures for special river basin restoration accordingly.

Unfortunately, we do not have enough data to evaluate the individual RRF facilities, including the botanical, hydrochemical, or hydraulic perspective; we only have information from the post-RRF period. As described in more detail in **Chapter 7**, it is very demanding to carry out reliable bioindication verification of the fact that the whole river basin or stream is favourable for the whole life cycle of the FPM, including the hyporheal phase of life.

So far, three RRFs have been built in the Czech Republic. All are regularly maintained by composting meadow management, which provides good quality FPM food. This type of management is discussed further in **Chapter 9**. On the other hand, part of the expectations associated with their construction was not fulfilled (significant long-term improvement of soil conditions and construction of a site with significantly better juvenile growth), or these effects are not accurately documented. Given the fact that more RRFs are currently being considered, it is necessary to critically assess the apparent construction mistakes caused by the construction companies or deficiencies caused due to imperfect knowledge, and learn from them. The current situation of some local populations is far worse than 20 years ago, and therefore we cannot afford to make further big mistakes.

Tests carried out to improve detritus quality showed, that slightly mechanically modified natural detritus was rated the best. The addition of benthos homogenate did not prove useful for juveniles originating from the Blanice River. The same applies for artificial food. Therefore, we recommend using proven natural food from sites as close as possible to sites where juveniles from semi-natural rearing will subsequently be planted.

Chapter 6: Methodology of freshwater pearl mussel rearing

Author: Mgr. Ondřej Simon, Bohumil Dort, Jaroslav Hruška
Reviewers: Ing. Karel Douda, Ph.D., Ing. Ondřej Spisar, Ph.D.

The focus of this part of the methodology dealing with FPM support is a detailed description of methods concerning rearing of freshwater mussels in the conditions of Czech localities. Brief review shows experience from abroad, as well as the gaps in knowledge from our country. The following is a detailed description of current breeding. It is also supplemented with the original description of the methods published by rearing founder, Jaroslav Hruška (Hruška 1999) – see **Annex 9**.

Rearing of FPM is one of the attractive and well-known parts of the Czech Action Plan (CAP FPM). This should not overshadow the fact that failing to revitalize the original habitat in a timely and sufficiently good manner means that there will be nowhere to plant the reared individuals. Another possibility is that these individuals will not be able to reproduce naturally after they reach adulthood. This applies for most, if not all, Czech localities. Thanks to rearing, disappearing populations may be artificially rejuvenated (and in several places this goal has already been achieved, see Simon et al. 2015); but this is far from sufficient to meet the main objective of the CAP FPM, which is the restoration of natural reproduction in FPM basins.

6.1 Current knowledge about rearing FPM

6.1.1 Short summary of selected *Unionidae* rearing papers

The rearing of the large *Unionidae* species, including the FPM, has been developed and conducted in North American conditions to improve the supply for the pearlescent pigment industry (e.g. Howard 1922; **Fig. 37**). A review of earlier works is provided by Araujo et al. (2015). Commercial rearing focused on pearl production (Sicuro 2015) developed in East Asia in the second half of the 20th century. Gatenby et al. (1996) published the first survey of breeds in the American environment, following a rather conservation point of view. Part of the work was a summary of the magnitude of growth and mortality of large bivalve molluscs. Strayer (2008) summarized basic knowledge on the ecology of freshwater bivalve molluscs in an extensive monograph.

The rearing of several large North American bivalve molluscs in separate circulation systems has been designed and successfully tested by Barnnhart (2006); another laboratory system using macrophytes is described by Kovitvadhi et al. (2008). Hyvärinen et al. (2020) tested pulse-through system, especially for FPM. In contrast to the most widespread rearing method in host fish species, the Thai authors introduced an in vitro method of glochidia cultivation of some bivalves using fish plasma (Kovitvadhi et al. 2006, Lima et al. 2012).

6.1.2 Short summary of FPM rearing in Europe

Buddensiek (1995) introduced pearl mussel rearing in perforated cages – primarily for bioindication purposes. Procedures for the so-called Czech method of semi-natural rearing (**Fig. 38**) were developed in the late 1980s and early 1990s by J. Hruška and his team (Hruška 1991, 1992a, b, 1999, 2001, AOPK ČR 2013a). Currently, mussel rearing takes place in several European countries (Preston et al. 2007). The latest review of mussel rearing was published by Gum et al. (2011). Lavictoire (2016) described rearing without the use of artificial feeding. For Austrian results of breeding programmes, see Scheder et al. (2014); Luxemburg results were published by Eybe (Eybe et al. 2013; Eybe et al. 2015); and results from Germany by Lange & Selheim (2011) and Denic (2018). Many results from rearing programmes are known only from conference presentations and are still waiting for detailed publishing (Geist 2010). Important negative results were published by Schmidt & Vandré (2010) and Taskinen et al. 2011).

In the Czech environment, Hruška (1992b) introduced the winter (shortened) and standard long (summer) rearing alternatives, with feeding based only on detritus obtained from selected springs (AOPK ČR 2013a). In winter, the activity of juvenile pearl mussels and fish in natural conditions is minimal. Therefore, this period of rest can be significantly shortened in rearing facilities by adjusting environmental conditions. To support summer and winter rearing, Jones et al. (2005) also exemplified two North American species of *Unionidae*. **Figures 39–42** show some selected results of the Czech CAP FPM (AOPK ČR 2013a).

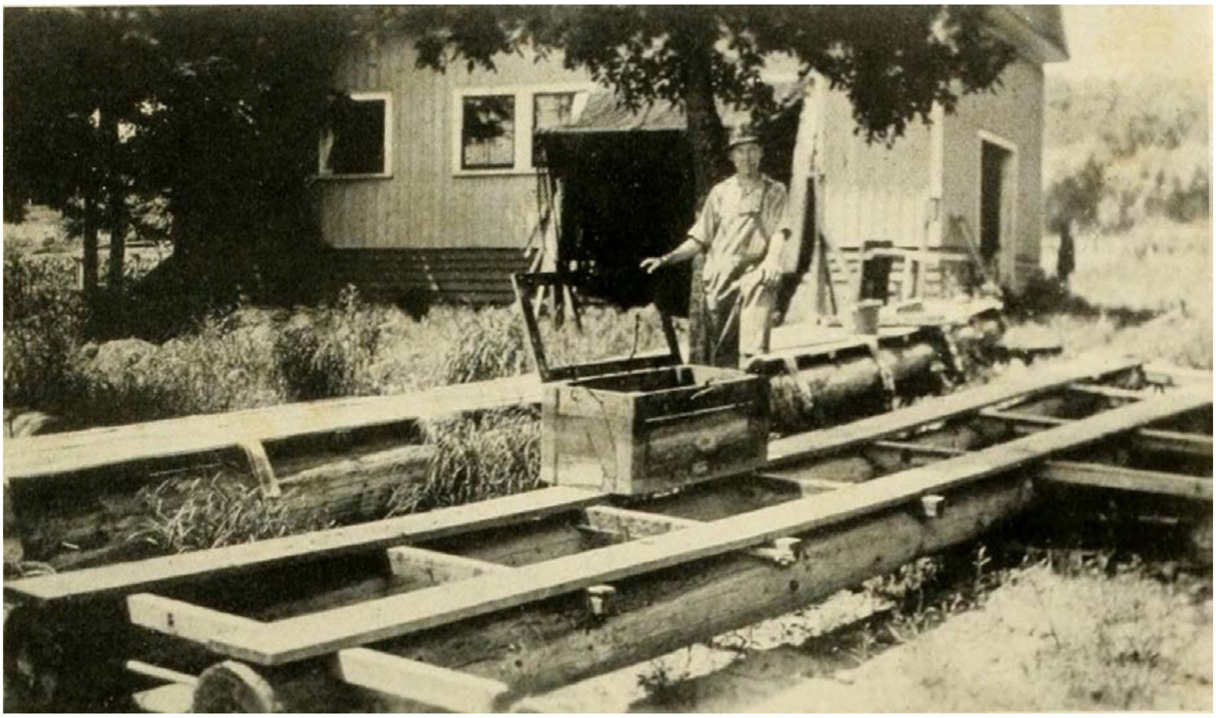

Fig. 37. Early rearing of large bivalve molluscs. The earliest well-documented efforts to breed large bivalves date back to the early 20th century in the United States (Howard 1922).

6.2 Food for pearl mussels and other bivalve molluscs in a natural environment and in rearing conditions

Detailed knowledge of the diet of juvenile stages of FPM is essential for both artificial and semi-natural rearing. However, studying microfiltration of food, such as by pearl mussels, is very difficult. Moreover, food intake is very complicated. Material sorting occurs both on the gills and in the sorting stomach. Thus, not all particles found in the gastrointestinal tract are used by bivalve molluscs to obtain energy or body-building substances (Bauer & Wächtler 2001). Therefore, it is necessary to use methods based on stable isotopes (e.g. C and N) to make it possible to determine which particle types are actually used.

With a sample of American bivalve molluscs, Nichols & Garling (2000) and Christian et al. (2004) verified that, although the bivalve mollusc alimentary canal is regularly filled with big algae cells, bivalve molluscs do not generate their biomass from algae but from bacteria or picoplankton (planktonic organisms smaller than 2 μm). Similar results for European species were obtained from Finnish lakes by Vuorio et al. (2007). In contrast, Newton et al. (2013) obtained different data based on isotopic analysis of a geographically wide sample of bivalve molluscs in North American rivers. Their study showed that about half of the food that bivalve molluscs use to build their biomass was algae.

The importance of algae in food may often be overestimated in other types of *Unionidae*. This is probably due to the easy identification of some (indigestible) algae and diatoms in the bivalve mollusc body, compared to amorphous detritus flakes or microscopically unidentifiable bacteria. A detailed study using isotopes for FPM is still missing. The literature states that FPM feed on detritus

Fig. 38. Rearing of bivalve molluscs in the Czech Republic: seven-year-old pearl mussels from Czech breeding led by B. Dort.

Fig. 39. Rearing result documentation of the CAP FPM. Big individuals from the control group held in a sand cage. Photo from a presentation by J. Hruška at the 2007 Action Plan evaluation conference.

Fig. 40. Control group individuals after 15 years of rearing, when they begin to reach sexual maturity. Photo from a presentation by J. Hruška at the 2007 Action Plan evaluation conference.

Fig. 41. Wild released individual after end the interstitial phase of life. Photo from a presentation by J. Hruška at the 2007 Action Plan evaluation conference.

Fig. 42. Individuals from the last phase of the first wave of rearing carried out by J. Hruška in 1998–2004, held in a control sand cage. Photo from a presentation by J. Hruška at the 2007 Action Plan evaluation conference.

(Bauer & Wächtler 2001), which is confirmed by the results of the Czech rearing of FPM fed only with detritus (Hruška 2004), see **Chapter 5**.

In practice, when it comes to rearing, we do not necessarily know exactly what bivalve molluscs eat and digest. It is sufficient to empirically verify which water or other material "has a positive effect on growth". Beaty & Neves (2004) were among the first to successfully test nutrition in *Unionidae* rearing using fresh river water. Using lake water for FPM is a similar method successfully used by the FBA in England (Lavictoire et al. 2016).

Some fish-keeping or common aquaculture practices using special compound feed were successfully adapted to FPM rearing. A team led by R. Neves gradually developed and tested the rearing conditions (substrate, circulation, algae feed, etc.) for several endangered American unionid species. However, many of these conditions are species-specific (e.g. Gatenby et al. 1997, O'Beirn et al. 1998, Jones et al. 2004, 2005, Hua & Neves 2007, Liberty et al. 2007, Hua et al. 2013) and the results are of limited use for the FPM rearing. Several teams have followed the R. Neves method during the rearing of FPM (Gum et al. 2011, Eybe et al. 2013). Commercially available mixtures (e.g. Nano-shell) optionally combined with detritus are commonly used to breed aquarium bivalve molluscs.

A rearing system using natural food and sturgeon as a host has been introduced by Araujo et al. (2003) to breed the southern European pearl mussel (*Margaritifera auricularia*). Currently, the rearing is being organized within project LIFE (Soler et al. 2015).

6.3 Releasing and monitoring subsequent survival of released bivalve molluscs

Bolland et al. (2010) focused on the description of localities and microhabitats where it is advisable to release bred FPM. It is appropriate to label the released individuals with visible optical marks (Araujo et al. 2015) or to use passive integrated transponders, PIT (Hua et al. 2015) so that they can be traced later (in the case of PIT also when under sediment) – **Figures 43** and **44** illustrates how poor the traceability of subadult pearl mussels is. So far, no data have been published abroad on the survival of released marked FPM individuals. Moorkens (2017) described successful detection of FPM juveniles after short-term captive breeding and after one year in the wild. Four juveniles were detected in the microhabitats in which they had been released.

Detailed results of the release of Czech rearings have not yet been published. A summary of the proportion of bred individuals was published by Simon et al. (2015). Marking of released individuals in the Czech Republic was tested only on a small number of subadults in the 1980s using aluminium markers. For the CAP FPM, standard plastic markers are used to mark adult individuals that have been handled, for example, during transfers or haemolymph sampling. So far, only finds of single marked individuals from Lužní potok stream have been documented (O. Spisar, pers. obs. – see **Figures 234** and **235** in **Annex 4**) or in Vltava River and Malše River (O. Simon, J. Horáčková pers. obs.).

Maximum growth rate does not necessarily mean the best option for conservation-motivated rearing (see **Chapters 5** and **7**). The growth rate is also determined by natural conditions in the individual parts of FPM area. Lowland populations (fast-growing, short-lived) and mountain/arctic populations (slow-growing, long-lived) are likely to show different results under optimal conditions.

Moreover, minimum rearing mortality does not necessarily mean a long-term advantage for survival in the natural environment. Hruška (1998) and Araujo et al. (2015) point out that rearing must imitate, as far as possible, the effects of natural factors in order to avoid the selection of individuals with adaptations that are completely unsuitable for survival in the wild (Jones et al. 2006, Hoftyzer et al. 2008). For example, in the specific natural conditions of Czech mountain rivers, individuals fed

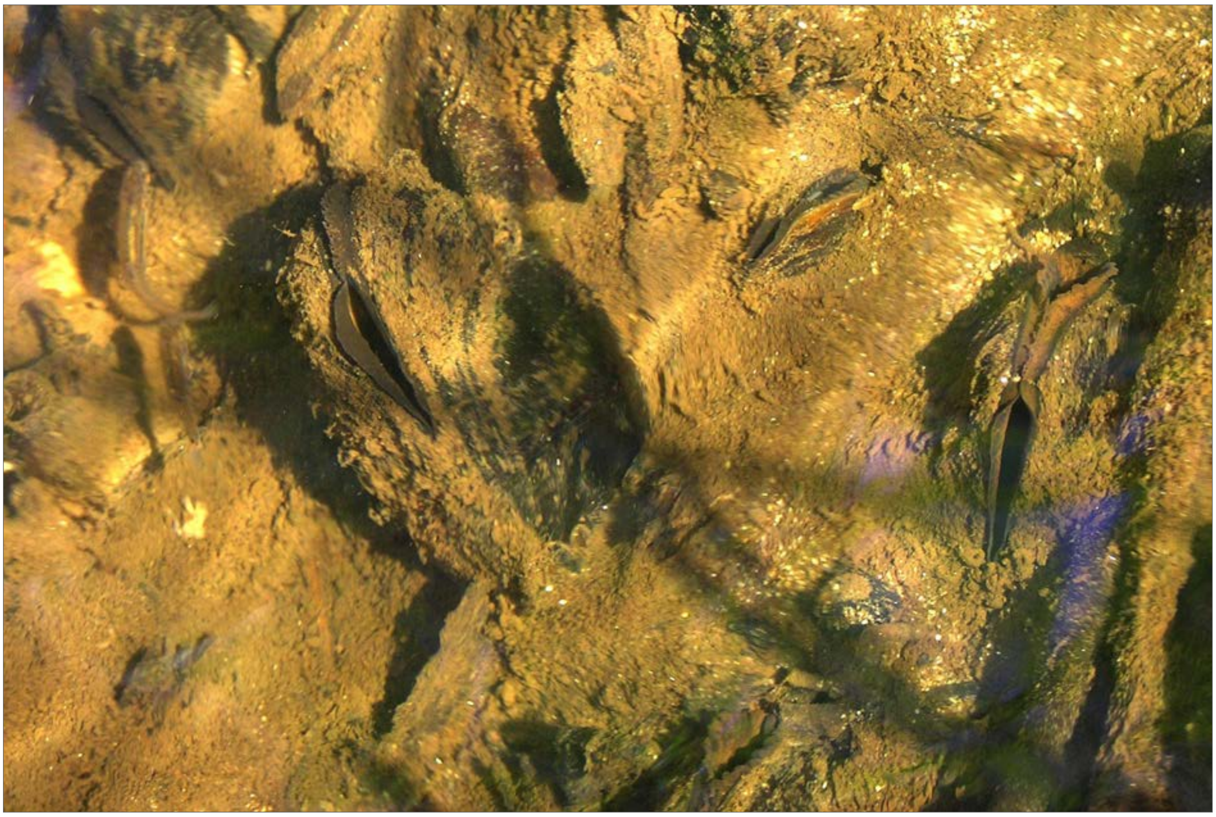

Fig. 43. Development of bred FPM released into the wild. After release, it was possible to observe gradually emerging subadult size individuals with significantly smaller excurrent and incurrent siphons in the Blanice River arm (photo from 2007).

Fig. 44. Development of bred FPM released into the wild. Size of individuals aged 5 and 6 years bred at the upper-temperature limit of the species in the upper part of the Vltava River basin within the second wave of rearing by B. Dort (photo from 2016).

algae-based high-nutrient feeds could have problems surviving in rivers with nutritionally poor food sources. This does not mean that intensive "quality feeding" in the early years of life is a wrong approach. However, such a procedure is not proven and individuals bred on this basis do not yet represent a cohort of fertile adults living in natural conditions. For example, the well-known conservation project for the pearl mussel population in the Lutter River was not based on rearing but on artificial fish infection combined with extensive river basin revitalization (Altmüller & Dettmer 2006).

The only exception are pearl mussels bred in the Czech Republic in recent decades, successfully found after being released into streams at several sites. Currently, the oldest bred FPM from the rearing carried out by J. Hruška's team have entered reproductive age, both in cage isolation (**Fig. 45**) and released into the wild (see the map of rejuvenated population localization, **Fig. 46**). Three local populations (Blanice River, Zlatý potok stream, and Rokytnice stream) have been rejuvenated to a significant extent. In the Malše River, small numbers of released juveniles were found in 2012 (Matasová et al. 2013), but recent monitoring confirmed only one individual after long extensive sand erosion processes (J. Horáčková, per. obs.). In Jankovský potok stream, where a small test sample was planted, the released juveniles were not found. When evaluating rearing results, it is, therefore, necessary to wait until the individuals complete the hyporheal phase of life and reach fertility.

Fig. 45. A – Development of bred FPM released into the wild. A small group of pearl mussels from the first rearing wave, held until the time of fertility in the sand cage, emitting glochidia (photo from 2016). B – Detail of FPM emitting glochidia (photo O. Simon)

6.4 Rearing procedure used in Czech Republic by Bohumil Dort, Gammarus company

6.4.1 Infecting host fish

For rearing, it is necessary to ensure in advance a sufficient number of fish for controlled infection of fish host gills. Based on experience, fish up to 15–20 cm from wild populations that have not yet come into contact with the pearl mussel (judging by the locality of origin) are suitable. Good results were also obtained with some fish hatchery lines (Douda 2015).

Fig. 45. B – Detail of FPM emitting glochidia (photo O. Simon)

In our conditions, *Salmo trutta* m. *fario* is a host of a parasitic glochidium larva (the glochidia size is 60×80 µm, **Fig. 47**). Due to the coevolution of both species, the bond between the FPM and the local trout subpopulation has stabilized (**Fig. 48**). Studies carried out in Norway (Larsen 2005) and in Germany (Altmüller & Dettmer 2006) have shown the highest success rate of parasitic infection in trout from the same river basin area as FPM, i.e. trout originating in the local sub-population. The risk of unsuccessful larvae development also increases with geographical distance between the FPM population and its host. Glochidia may also adhere in other hosts, such as the gills of *Oncorhynchus mykiss*, but die within 48 hours (Scharsack 1994).

Unfortunately, trout populations in the Czech Republic were often mixed and replanted between source basins. Thus, in our conditions, host compatibility is not always geographically linked. There is also significant variability in host quality across populations. It is therefore always advisable to verify the quality of the hosts used for the successful course of the parasitic phase (Douda 2015).

Great attention must also be paid to the origin of the glochidia used for infections to maintain genetic variability of the reared animals (Hoftyzer et al. (2008) deal with the genetic aspects of *Unionidae* rearing). It is preferable to use a glochidia mixture of multiple individuals inseminated in natural colonies, preferably of 15 naturally inseminated females from a large colony. From this perspective, long-term closed laboratory systems may be questionable – only a small number of sperm and egg producing individuals are involved in reproduction.

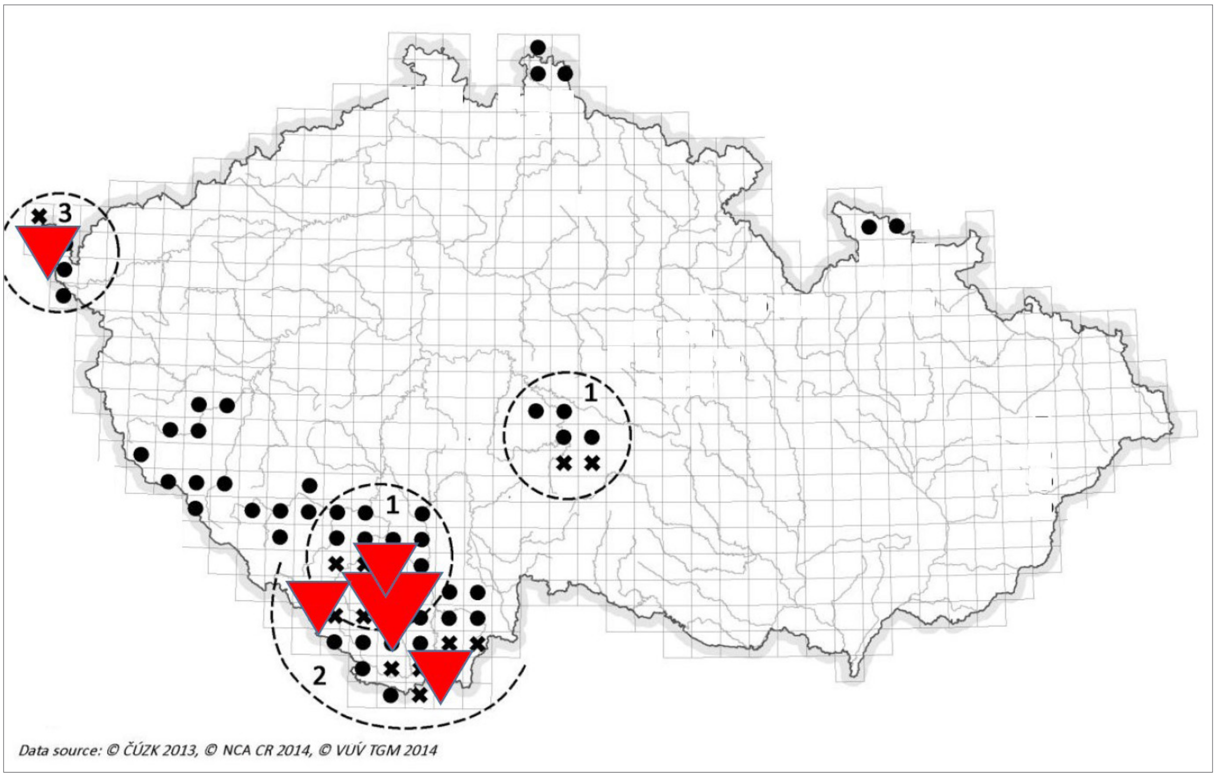

Fig. 46. Localization map of populations rejuvenated by semi-naturally bred juvenile mussels. Large triangle – hundreds of recorded subadults after completed hyporheal phase of life; small triangle – only small number of recorded subadult individuals. It should be noted that a number of individuals in cold locations have not yet completed the hyporheal phase of life. Black dots – documented historical occurrence; crosses – occurrence after 1985. The numbers indicate genetically detected local populations: 1 – Blanice population, 2 – Malše population; 3 – Saale population, with indicated recent range. Modified after Simon et al. 2015.

Infection procedures used by J. Hruška (a glochidia mixture obtained from many naturally living individuals or cage infection downstream from natural numerous colonies) and subsequent rearing under the best possible natural conditions were successful in this respect (Hruška 1998). A recent study focused on genetic aspects of FPM populations in the Czech Republic (by Bryja et al. 2010) has proven that their genetic variability has not been reduced. The evidence is good genetic variability of obtained subadults.

Other rearing practices depend on the sum of the average daily water temperatures of the stream where the FPM are found, starting at the beginning of the vegetation season (April). Spontaneously maturing fertilized eggs released by pearl mussels are collected to determine their degree of development correlating with the sum of temperatures. The glochidia maturation time can be roughly determined according to weather development.

In southern Czechia, glochidia mature mostly in August, at the peak of summer. Mature glochidia are collected to control the infection of fish hosts in a prepared bath. The concentration of glochidia in the bath is tens of thousands per litre of water.

6.4.2 Rearing of infected fish – controlled metamorphosis of pearl mussels

The following is an example of a precise timing procedure for controlled metamorphosis of FPM, which depends on the hydrological conditions of the site.

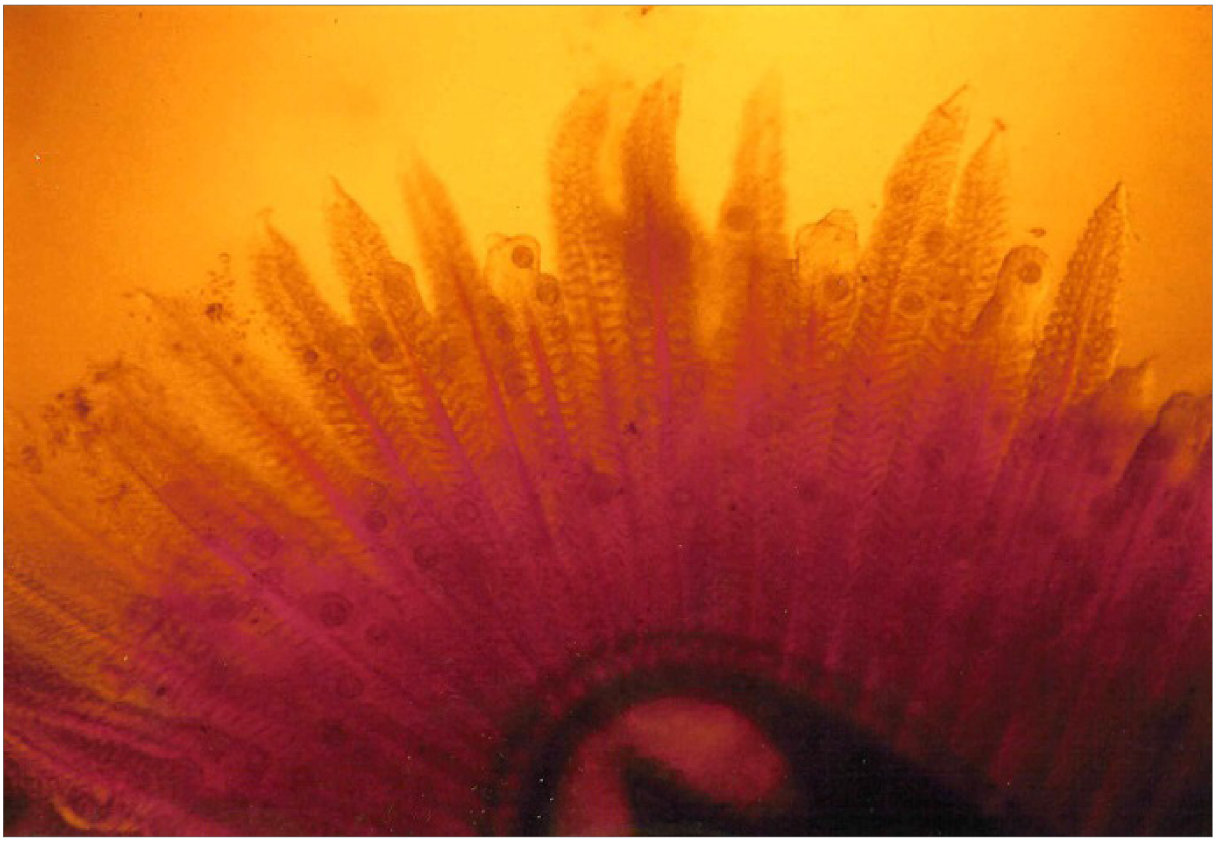

Fig. 47. Pearl mussel glochidia visible as dark dots on the gills after acceptance by the fish's immune system and fully surrounded by healing tissue.

Following controlled infection, the fish are kept in hatchery troughs, ponds, and raceways or cages with a sufficient supply of food and oxygen. Fish designated for spring rearing are thus kept until the next growth period. Fish designated for winter rearing are kept in the natural environment until the beginning of the off-season (October).

Controlled metamorphosis can be initiated at any time in the period following host infection with glochidia until the natural encapsulation of juvenile FPM. It is possible to shorten this period according to the sum of temperatures before the start and the sum of temperatures after the start of controlled metamorphosis. Thus, at Czech localities, it can be started at any time from August, when the fish are mostly infected, to June or July of the following year, when metamorphosis in the host ends naturally. More detail about this issue is to be found in **Annex 9**.

About 60 days are necessary during winter rearing to complete the metamorphosis and encapsulation of pearl mussels, while only about 30 days are necessary during spring rearing. Of course, the stages depend on the sum of temperatures prior to encystment.

During winter rearing, from October to the end of February of the following year, not only themetamorphosis is completed but also the first growth period. Healthy individuals reach a shell length of 1 mm on average. After a rest period in the cage situated in flowing cold water, at the beginning of the growing season, the second growth period (1+) begins. During one year, juvenile FPM undergo two growth periods without adversely affecting their further development and survival. If there is no rest period between the growth periods, delays in growth of varying lengths occurs and, if growth continues, FPM mortality also occurs (Hruška 1999). An important advantage of winter rearing is the acquisition of juvenile FPM of age 1+ at the beginning of the growth period, when bioindicator evaluation of the habitat is carried out.

Fig. 48. *Salmo trutta* m. *fario*, the host of FPM glochidia in Czech conditions. The larvae develop well in young 1–2-year-old trout; older fish (in the picture see a mature animal) are usually immune to glochidia. Photo: David Štrunc.

The advantage of spring rearing is the short period of temperature-controlled glochidia metamorphosis in the host, due to the accumulation of daily degrees before being placed in rearing facilities during the off-season. Since pearl mussels enter the first growth period (0+) at the beginning of the growing season, they cannot be used for bioindicator evaluation. However, the advantage is the less laborious continuation of pearl mussel rearing, namely by cage rearing under natural conditions (**Chapter 6.4.3.3**). As for the further survival of FPM resulting from both types of rearing, research is only at its beginning and results cannot be precisely proven yet.

6.4.2.1 Winter rearing

At the beginning of the off-season season, fish are transferred to rearing facilities (water tanks with recirculation or water exchange, **Fig. 49**) at the Rearing and Reproductive Feature (RRF), followed by temperature-controlled metamorphosis of glochidia to pearl mussels. This varies according to the type of RRF. Basically, the emphasis is laid on keeping the water and water tanks clean and keeping the water at a constant temperature of 15–17 °C to ensure a smooth metamorphosis progress. During rearing, the fish are regularly fed and, of course, there is sufficient oxygen saturation of water.

After reaching about 700 daily degrees (D° – the sum of the average daily water temperatures measured in rearing facilities), the first excysted (mostly dead) juvenile FPM are usually found. At this time (i.e. at the beginning of November) it is necessary to start search for them regularly in impurities

Fig. 49. Fish rearing facility. A recirculation rearing system made by K. Douda as a part of the EU project "Coexistence of humans and pearl mussels in the Vltava floodplain", used by Šumava National Park Administration for FPM rearing.

(sediment at the bottom) collected on a sieve with a mesh size of 0.1 mm. When the values reach about 900 D°, the intensity of juvenile excystment from host tissue increases. Still, the excysted individuals are rather small and underdeveloped, about 0.25 mm in size. As the sum of temperatures increases, more viable individuals are obtained. Juveniles larger than 0.3 mm excyst from host tissue when the 1000 D° is exceeded. Above 1,200 D°, the excystment intensity reaches its peak, and juvenile FPM bigger than 0.5 mm are often found. When the level of 1,400 D° (mid-December) is reached, the number of excysted pearl mussels decreases sharply until controlled metamorphosis is complete. The water in the rearing facilities is gradually cooled so that the temperature fluctuations do not exceed 5 °C within 24 h. When the temperature reaches the surface water temperature, the fish are returned to the care of the cooperating fishing organization by agreement.

Based on the repeatedly confirmed experience gained during controlled metamorphosis, a 15 cm host fish can easily feed 1,000 encapsulated glochidia until they are converted to juvenile FPM (**Fig. 50**).

6.4.2.2 Spring rearing

At the beginning of the growth period, the fish are transferred to rearing facilities in the rearing station (water tanks with recirculation or water exchange), followed by temperature-controlled metamorphosis of glochidia to pearl mussel. The process is the same as during the winter rearing. As mentioned in **Chapter 6.4.2**, the time required for metamorphosis is reduced by daily degrees accumulated especially during the end of summer and autumn.

6.4.3 Rearing cycle of juvenile pearl mussels after J. Hruška

The Czech method is a whole complex of procedures, including food care, bioindicative evaluation of juvenile FPM responses to individual conservation methods, and resulting adjustments to the procedures used. For more details see **Annex 9** (semi-natural rearing) or the work by Hruška (1999). The following text focuses on working procedures of semi-natural rearing.

6.4.3.1 Winter rearing

When metamorphosis on the host is completed, 450–500 individuals are stored in rearing detrital cages (**Fig. 51**). These are 1-litre plastic containers with a lid. The containers are kept in the laboratory at a constant air temperature of 16–18 °C, with a slot between the lid and the container for oxygen access to the aquatic environment.

Semi-natural rearing involves periodic cleaning of rearing detrital cages, thorough inspection of the reared individuals and feeding. The interval for each cage is set to 120 hours (**Fig. 52**). After this time, the contents of the detrital cage (i.e. water and detritus with kept FPM) is strained through a sieve with a mesh size of 0.2 mm. The mesh size of the sieve gradually increases as the mussel shells grow. A sieve with a mesh size of 0.3 mm is used at the end of the rearing. Each pearl mussel is placed in a Petri dish to verify the rearing process using a binocular stereomicroscope and other magnifying techniques (**Fig. 53, 54** and **55**). Dead individuals are removed from rearing. In the case of the slightest hint of pearl mussel disease, the suspected specimens are quarantined. The cleaning period for the affected rearing detrital cage is shortened to 24 hours, in case of the fungal disease to 12 hours. Large and active specimens are usually free from bacterial infections or resist the infection for a long

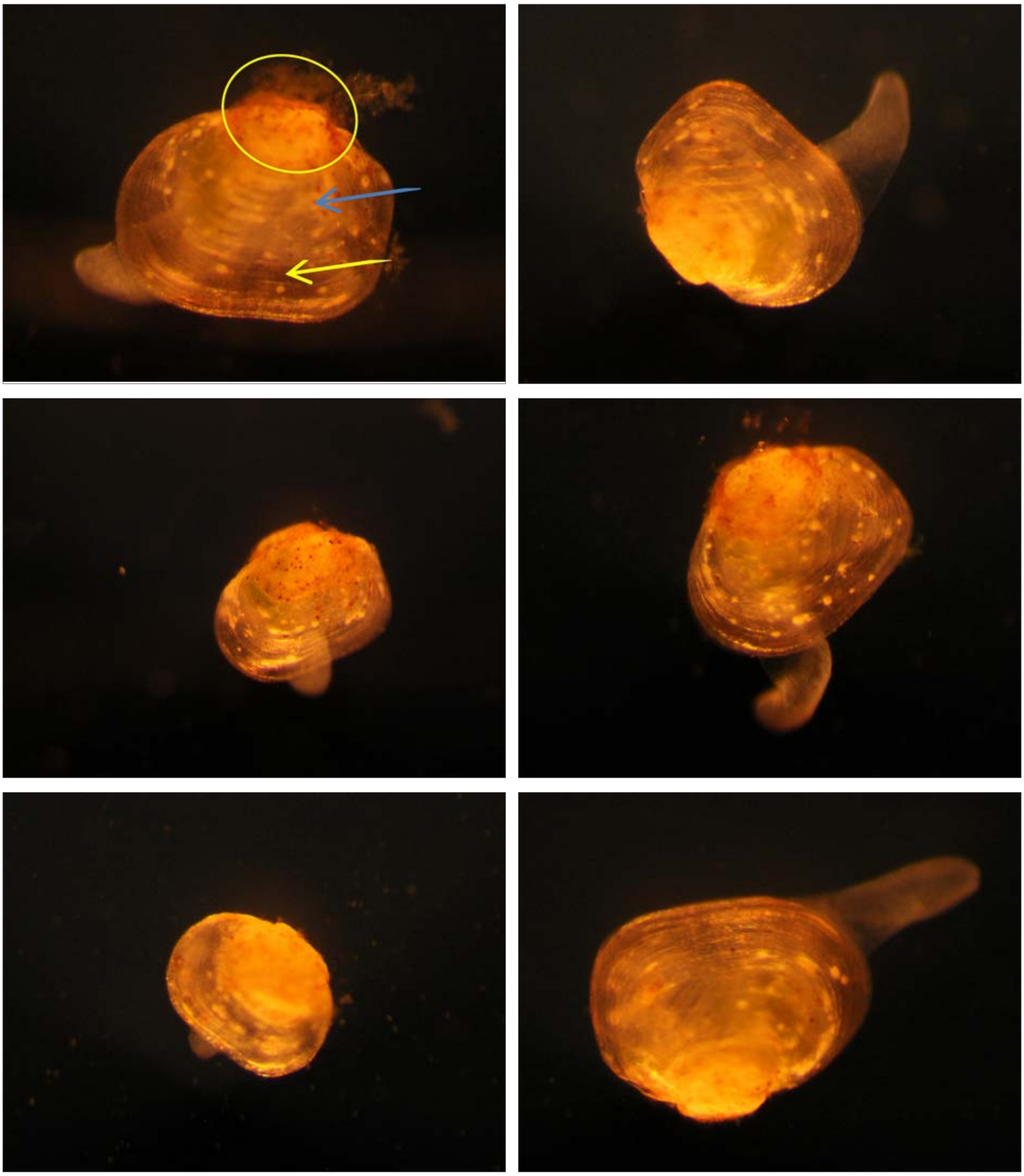

Fig. 50. Juvenile pearl mussels. The variability intypical size and shape of juvenile pearl mussels at age 1+ in comparison with age cohort 0+. A glochidial cap in the first photo (yellow circle – the oldest opaque part of the shell with the red growth that corresponds in size to individuals 0+), part of shell growth that corresponds to the first growth period (blue arrow – the milky coloured part), and the lightest part of growth that corresponds to the beginning of the second growth period (yellow arrow). 36c-f shows variability of length of 1+ mussels from the Blanice population.

Fig. 51. Rearing detrital cage after Hruška (1999) with a breeding capacity of up to 500 FPM individuals.

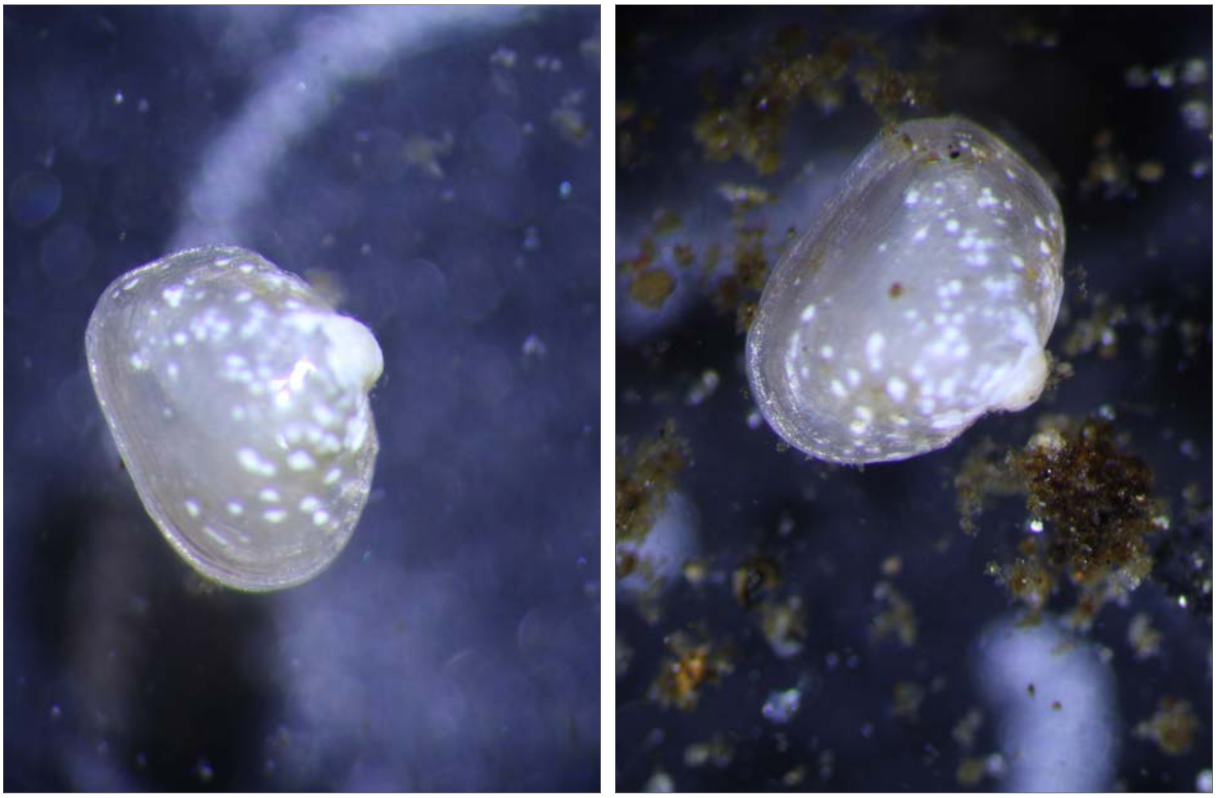

Fig. 52. Example of juvenile shell increment after a 10-day period. Juvenile individual measuring 1400,59 µm (on the left photo) and the same individual after 10 days measuring 1442,34 µm (on the right photo) – a difference of 41,75 µm (June 2023).

Fig. 53. Rearing of juvenile FPM. In the rearing detrital cage, juvenile FPM are gathered under detritus, forming groups (photo was taken after the detritus was partially blown off).

Fig. 54. FPM during periodic inspection under a microscope.

time. However, if the fungal infection is not detected, mortality can be up to 100 % over a 120-hour period.

Juvenile pearl mussels are fed with detritus taken from selected springs or small rills connected to main stream. Thus, bivalve molluscs are exposed to changes in water chemistry to replicate the changes in flow and also gain immunity to infections.

The rearing phase, when the growth period is induced under laboratory conditions, is completed at the end of February. The air temperature where rearing detrital cages are stored is gradually lowered by 5 °C in 24 hours to a value between 1–4 °C. FPM which are to be used for bioindicator tests are further stored in laboratory conditions until the beginning of the vegetation season, which induces the quiescence phase of the winter period. However, periodic inspection of FPM, including feeding, continues during this period. The remaining FPM are stored in the sand cages that are kept under natural conditions within the cage rearing.

The winter type of rearing is mainly used to obtain FPM for bioindicator evaluation to be performed in the following vegetation season. Individuals undergo evaluation at the beginning of their second growth period (second year of life, i.e. 1+). The same starting conditions for all specimens resulting from rearing determine the quality of the evaluation performed. Bioindicator evaluation is carried out in cages at selected locations.

6.4.3.2 Spring rearing

This type of rearing follows the same procedures as winter rearing. When metamorphosis is finished, FPM are stored in rearing detrital cages in the laboratory at air and water temperature ranging from 16–18 °C. The following phase is at maximum one-month long period of semi-natural rearing while waiting for good hydrological conditions in the rivers (after the spring thaw); rearing continues in natural conditions in sand cages (see the following chapter).

6.4.3.3 Cage rearing of juvenile pearl mussels 0+ to 5+ in the stream

Sand cages or "Hruska cages" (see Bílý et al. (2018) for detailed picture) have been developed in recent years to rear FPM in natural conditions (by J. Hruška, **Fig. 56**). It is a plastic container with a tight lid of dimensions 170 × 100 × 70 mm. It is necessary to adjust the cage, that is to make large openings on all walls and the lid. These openings are covered with a mesh fabric (e.g. Uhelon) with a mesh size according to the age of FPM to be kept in the cage (ranging from 200 to 2000 μm). Sand with a fraction size of 2–4 mm is placed in the cage up to 1/3 of its height. The microhabitat in the sand cage is very similar to natural conditions on the stream bed and allows the introduction of water-borne detritus through the perforated walls of the cages.

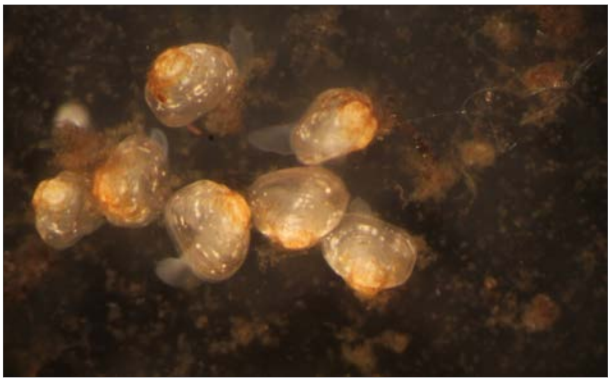

Fig. 55. Detail of juveniles 1+ suitable for bioindicator application, and surrounding fine detritus.

The pearl mussels are stored in sand cages in quantities depending on their age (and hence shell size), usually from 1,000 specimens 0+ up to 100 specimens 5+. Safe and proven sites are selected in advance to eliminate adverse natural conditions (floods, drifting ice, etc.) and anthropogenic impacts. Locations are selected that imitate as much as possible the natural habitat of FPM, namely sections with slower water flow (up to about 20 cm/s) at the meander or bend on the inside. The cages are provided with anchoring stone before being placed in the water. The cage is fastened to a flat stone weighing at least 1 kg using a net with a mesh width of 10 × 10 mm; the cage is placed on the stream bed so that the longer side of the cage and the stream form an angle of approximately 45° (**Fig. 57**).

During cage rearing, the cage is regularly checked for integrity and captured sediments are cleaned. The inspection and cleaning interval is set at 14 days, but is shortened at low flow rates or immediately after high flow rates. When checking the cage, the surface of the mesh fabric is first cleaned with a soft brush and the fine sediment is washed out by circular movements. Everything must be done under water.

At the end of the growing period, the cages are taken from the rivers to perform an annual assessment of rearing success (**Fig. 58, 59**), that is, identifying average shell increments and mussel survival. When evaluating or replacing a cage, its contents are poured into a circular container partially filled with water. Subsequently, circular movements are used to wash out lighter particles into a second container. This method is also used in panning for gold, where it is in contrast desirable to obtain heavy particles. After one rinse, it is necessary to sort the ballast made of fine sediments, *Trichoptera* larvae (caddisflies), *Chironomidae*, and benthos, including their secretions. Moreover, *Trichoptera* larvae are thoroughly inspected due to the possible incorporation of live pearl mussels (0+, 1+) into their walls.

At the end of each growth period, the rearing cage is replaced with a cage with a thinner mesh.

Fig. 56. Sand cage for juvenile FPM – a cage with sand, equipped with an anchoring stone and net. Below: a cage during unloading of pearl mussel from rearing detrital cages to sand cages.

6.5 Measures to prevent mixing of reared populations

When breeding genetically different populations (such as the Saal population originating from the Lužní potok stream and bred in the Blanice River), it is always necessary to include separating objects behind the cage rearing place in river arms of streams, because the cage kept in open water can easily be damaged by an extraordinary events like floods. Juvenile stages could then enter the habitat of a genetically different population of FPM. For this purpose, already in the beginning of rearing, sedimentation pools with a regime of drying and freezing were established behind the rearing sections, which prevented any escaping juvenile stages from entering the stream. It is therefore preferable to implement sand cage rearing in the mother river basin. This procedure can also better prepare juvenile individuals for all the environmental factors they may encounter after release into the wild and the nature of their fluctuations (sudden changes in chemistry during melting, current changes, locally occurring pathogens, nature of carried sediments, etc.). No genetical contamination was detected in 2014 (Simon et al. 2015) or in more recent sampling (Douda 2019 unpublished data).

Fig. 57. A sand cage placed in the field immediately after installation (compare with a clogged cage in Figure 69).

Fig. 58. Five-year-old FPM bred in sand cages in the Malše River.

6.6 Verification of the applicability of "Missouri stones" (mussel silos) for rearing freshwater pearl mussels

Fig. 59. A juvenile FPM bred in "Missouri stones" in the Teplá Vltava River in 2016. Clearly visible are the latest light incremental stripes, whitish older increments, and a muscular leg.

To verify the possibility of using "Missouri stones" for FPM breeding, two short experiments were done. Mussel silos ("Missouri stones" or passive flow cages) were made according to examples from the M.C. Barnhard method (unpublished; **Fig. 60**). The inner perforated cylinder was made of plastic with a stainless-steel sieve at the top and bottom. Before use, the interior was lined with 350 µm Uhelon fabric. The pearl mussel individuals were placed in these silos in the Teplá Vltava River for one month; Buddensiek plates were also installed. The average survival after 30 days was 84.5 % (2 silos per 7 juveniles of 1+ cohort, 0.8–1.6 mm long) versus 95.8 % for pearl mussels in parallel-installed cages. (4 Buddensiek plates per 7 juveniles of 1+cohort, 0.7–1.8 mm long) (Tichá et al. 2020).

Removal of juveniles from the mesh proved to be impractical as there was a great risk of losing individuals due to the opacity of the surfaces. A better option seems to be covering the outer stainless-steel grid with Uhelon mesh or directly using a fine stainless-steel mesh for the construction of the inner basket.

6.7 Planting and survival monitoring of reared juvenile freshwater pearl mussels

The planting of reared pearl mussels has been taking place in the Czech Republic since the early 1990s. So far, old bred individuals have been planted in significant quantities in the Blanice Nature Reserve (**Fig. 61**; 49,468 individuals in total since 1995) Other amounts of planted individuals are as follows: Teplá Vltava River 1,180; Zlatý potok stream 887; Lužní potok stream 1,329; Malše River 438. A small control group of 42 individuals was planted at an unpromising site on the Jankovský potok stream and 34 individuals in the Bystřina stream (Simon et al. 2015). The size of released individuals does not allow use of fixed markers on the shells since the older layers of the shells rapidly dissolve **(Fig. 62)**. However, survival monitoring is possible in all populations due to the long-term absence of natural reproduction at most sites. At sites where natural reproduction is confirmed in the very low number (lower course of the Malše River, Blanice River millrace in the town of Husinec), limited reproducing populations are located downstream, although they were released dozens of kilometres upstream. In the Teplá Vltava River, where bioindication testing confirmed good conditions for juveniles, subadults and shells of dead subadults were found (2012–2020) in the areas of mussel release carried out in 1998 (Matasová et al. 2013; **Fig. 63** and **64**). The observed number of reared subadult individuals has been growing year-on-year and, in some sites, it has already reached 10 % of the total population (Simon et al. 2015).

Fig. 60. A prototype of a so-called "Missouri stone" used for experimental purposes.

Fig. 61. Detail of a subadult individual with completed interstitial phase of life with still well-visible increments (successful result of rearing and subsequent planting in the Blanice River).

Fig. 62. Comparison of the size of the eroded part a shell of pearl mussel bred in the 1990s with the approximate maximum size of planted FPM (in the same scale).

Fig. 63. A clearly visible cohort of subadults planted in the Teplá Vltava River in 1998. Shell of a subadult individual found in 2012 below the release sites.

Fig. 64. Shell of an adult from the same site like in Fig. 63.

Chapter 7: Bioindication methodology

Authors: Mgr. Ondřej Simon, Ing. Kateřina Rambousková, Bohumil Dort, Mgr. Michal Bílý, Ph.D. Jaroslav Hruška

Reviewers: Ing. Karel Douda, Ph.D., Mgr. Jan Švanyga

If young individual freshwater pearl mussels (FPM) in meshed breeding chambers or other containers are exposed to controlled experimental conditions, it is possible to determine how quickly they have grown and, if necessary, to evaluate their mortality. Since the beginning of the Czech Action Plan for freshwater pearl mussel (CAP FPM) (Hruška 1992b, 1999), these bioindications have been among the main tools used to study the ecology of the species, to identify critical life cycle periods, and to verify new methods to improve their habitat.

This part of the book summarizes how to perform or evaluate bioindications, and to contribute in general to standardization of the procedure. In addition, we list several recent procedural improvements that can make bioindications cheaper and can facilitate statistically correct result evaluation. When explaining the details, we refer to other chapters of the methodology concerning breeding and food supply improvements. This methodology should complement the methodological documents of the CAP FPM (AOPK ČR 2013a), the wording of which we often refer hereto. The text is available at https://www.zachranneprogramy.cz/en/freshwater-pearl-mussel/.

7.1 Brief theory and scope of application of bioindication method concerning FPM breeding

The general principle of various bioindication systems is the assumption that environmental conditions reflect both the occurrence and fitness of individuals of each species, higher taxa, or ecological group. A European bioindication school developed in hydrobiology in the second half of the 20th century (Zelinka & Marvan 1961, Liebmann 1962, Sládeček 1973). Its members have developed and established several saprobiological bioindication systems that are used, among others, to evaluate the degree of watercourses eutrophication (e.g. the current Czech standard ČSN 75 7716). However, this approach has been widely criticized and has not made much of an impact in the Anglo-Saxon world (Allan & Castillo 2007, Lamberti & Hauer 2017). The occurrence of organisms is influenced not only by physico-chemical environmental conditions, but also by interspecies relationships, life history, population cycles, trophic cascades, and other factors (Allan & Castillo 2007, MacNeil & Briffa 2009, Heino et al. 2015).

It is, therefore, more appropriate to use bioindications in the narrow sense, namely to use the exposure of a small sample of a certain species in a given environment to conclude whether environmental conditions suitable for this species could be expected. In this case, it may not be a problem if we are not sure which factors affect the species. In addition to mortality rate, we can also obtain data concerning growth rate, fertility, or other fitness characteristics of a species, especially when studying long-lived organisms. The principle is similar to well-established "transplantation" experiments in botany (Grace & Wetzel 1981).

As part of the CAP FPM, the bioindication method first developed by Buddensiek (1995) (**Fig. 65**) is used to monitor the ecological demands of freshwater bivalves. This method is based on accurate measurement of the size of fast-growing juveniles at the beginning end of the experiment. Grow rate is then evaluated at individual sites. This method also provides mortality data; for example, increased mortality rate can indicate acute environmental toxicity.

The method was also adopted and used repeatedly by Hruška (Hruška 1995, 1999, Hruška & Volf 2003). Due to the high mortality rate of individuals just after excystment (cohort 0+, i.e., individuals who have not yet completed the first growth period), Hruška was the first to use individuals at the end of the first growth period (i.e., at the end of the first winter – cohort 1+) in bioindication tests.

The applicability of the bioindication method in the specific conditions of Bavarian streams and the use of individuals aged 0+ have been criticized by Schmidt & Vandré (2010). Gum et al. (2011) specified the conditions under which the method could provide good results. They also provided an overview of the use of Buddensiek plates in other European countries. However, the use documented by the authors focused mainly on breeding, less on bioindications.

The possibility of shortening exposure time from a typical period of 3–9 months (Hruška 1999, Denic et al. 2015) to one summer month while maintaining benchmarks was introduced by Douda et al. (2012). They pointed out inter alia the fundamental relationship between temperature and growth rate in FPM development, previously documented Hruška (1992a). A fundamental study comparing the survival of populations of individuals aged 0+ in the Rhine, Elbe, and Danube River basins at the end of the first winter was published by Denic et al. (2015). This work points out the relationship between growth and survival and the initial size of mussels. The biggest increases were shown in exposed individuals in one of the tested sites where natural reproduction still took place. The longitudinal profile of a mountain river was also evaluated, with biodindications performed with Buddensiek plates and other methods (Černá et al. 2017).

In addition to Buddensiek plates, it is also possible to use other open breeding systems to perform bioindications. Plastic cages filled with sandy substrate – **Hruška sand cage** (Hruška 1999, Bílý et al. 2018) or **mesh tubes** – net cylinders made of stainless steel mesh – are used for group rearing of individuals (Pasco et al. 2015).

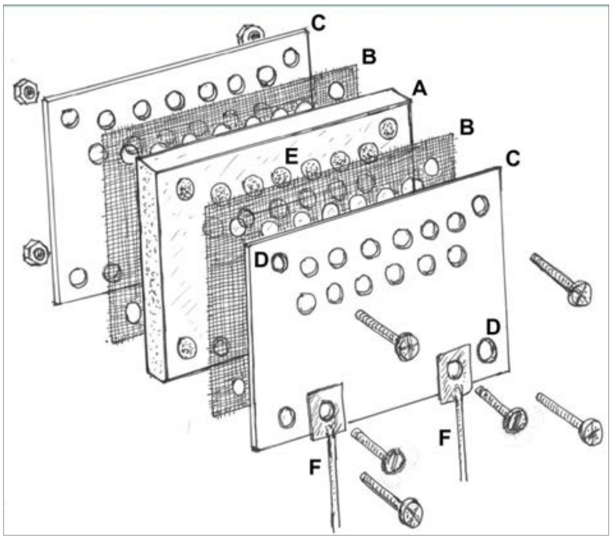

Fig. 65. Buddensiek plate with a set of small chambers for juvenile mussel individuals. A – inner part (sheet thickness ca. 9 mm) with drilled breeding chambers (Ø 6 mm, E), B – fine nylon mesh (Uhelon 200 µm), C – thin (2 mm) plastic sheets allowing attachment of Uhelon to inside, drilled breeding chambers and other holes (D) to insert fasteners and steel pins (F) used for cage installation in the current– see Bílý et al. (2018) for technical drawing. Illustration: Michal Bílý.

Activities under the CAP FPM (AOPK ČR 2013a) use procedures designed by Hruška. They involve breeding juveniles aged 0+ and above in sand cages, installed directly in streams with a suitable detritus composition (e.g. Teplá Vltava River; B. Dort – pers. comm.). These systems can also be used for bioindications. Černá et al. (2017) present the use of a large number of Hruška sand boxes (100 individuals per box) for bioindication evaluation of the **longitudinal river profile**, or dozens of boxes (100–2,000 individuals per box) to compare **microhabitat conditions** at three sites. For all bulk systems, it is only possible to perform a summary evaluation of the average increment. Another option is to use selection of the largest individuals to reduce the impact on the mortality rate increase (largest individuals have lowest mortality), as found by Černá et al. (2017). Detailed description of using Buddensiek plates and Hruška sand cages was recently published by Bílý et al. (2018).

Using of open cylinders, made of stainless steel mesh – **mesh tubes** (Bílý et al. 2020), developed for bioindication on 1+FPM juveniles in shallow hyporheal is not described in this book in detail. This new method was recently tested for ethological studies on FPM juveniles and it is not usually used for routine bioindications.

To summarize, the described bioindications can be used within the CAP FPM, especially in order to evaluate the effect of all factors on juvenile individuals exposed for some time at a site, as described above ('IN-situ bioindication'). On the other hand, it is possible to keep individuals in a laboratory under constant conditions and evaluate, for example, the detritus quality ('EX-situ bioindication').

In the CAP FPM, the EX-situ method of bioindications was introduced by Hruška (AOPK ČR 2013a). This approach has not been used often by other teams (except for farm tests carried out in Luxembourg, Eybe et al. 2013). EX-situ bioindication is a method of a direct evaluation of the detritus carrying capacity – food of early (and adult) FPM stages. The main advantage of this method is elimination of the considerable impact of temperature on the growth of juveniles and detritus carrying capacity (Hruška 1992a, Douda et al. 2012). There is still relatively little information on biological, chemical, or microbial detritus characteristics (Zimmermann-Timm 2002, Wotton 2007, Černá et al. 2017). Detritus from Šumava Mountains helocrenic springs was microscopically analysed by Tichá et al. (2012), and its biota was described by Kubíková et al. (2011, 2012). The typical feature of this detritus is high complexity, variability, and seasonal changes in composition (Tichá et al. 2020), for more details about detritus see **Chapter 5**. Comparable data for river detritus from other countries are still lacking. As part of proposals concerning restoration measures for catchments with FPM, this method of EX-situ bioindication tests was used in the Czech Republic (AOPK ČR 2013a), as well as in Germany and Austria.

In the following text, work procedures are presented separately concerning complex bioindications on site using the IN-situ method and laboratory tests in controlled conditions by the EX-situ method.

7.2 On site bioindications (IN-situ)

7.2.1 Methods and procedures

7.2.1.1 Materials and equipment

In pursuance of bioindications, juvenile FPM in the earliest life stages (age cohorts 0+ and 1+) are separately stored in Buddensiek plates (**Fig. 65, 66, 67**). The bioindication unit (125 × 85 mm) consists of three perforated acrylic sheets. The resulting holes (Ø 6 mm) then represent breeding mesh chambers for individual juvenile mussels. The inner part of the plate (9 mm thick acrylic sheet) is covered on both sides with fine nylon mesh (Uhelon 200 μ), which prevents juveniles escaping from the breeding chambers. The inner body of the acrylic sheet with Uhelon is then covered on both sides with thin acrylic sheets (2 mm). All parts are bolted together with four stainless steel bolts and nuts. In the lower side of the plate there are another two holes – these are for inserting steel pins when the plate is installed in the field (Bílý et al. 2018).

The Hruška sand cage is made from a plastic food storage container (PET material), measuring about 15 × 20 × 7 cm (**Fig. 68, 69**). All sides of the container have a window covered with nylon mesh. The mesh size must be as large as possible to allow penetration of organic particles, but at the same time preventing juveniles from escaping (200–300 µm is used to breed a cohort of 0+ individuals). The container is filled with washed and sieved coarse sand from a non-calcareous oligotrophic stream. For individuals of age cohorts 0+ and 1+, a grain size of 1–2 mm is used (Bílý et al. 2018).

7.2.1.2 Equipment installation, inspection, and collection

The Buddensiek plate is installed and attached to the sandy-stream bed with two steel pins. A data logger records temperature and light (**Fig. 66**). When the stream bed is too hard, a stone with metal handles is used. **Figure 67** shows a long-term installed Buddensiek plate with captured fragments of macrophyte vegetation.

The Hruška sand cage is attached with a 10 mm mesh net to a flat stone weighing at least 1 kg (**Fig. 69**). A hook is used to install the sand cage in a suitable place on the stream bed with well-flowing water, so that the longer edge of box forms an angle of about 45° with the thalweg. When placed at the stream bed level (the top level of the sand in the sand box is at the same level as the stream bed), the fixing stone is placed from above.

All breeding and bioindication equipment used must be maintained and cleaned to ensure proper operation. Frequency of cleaning affects the micro-conditions in the breeding facilities and can thus affect the health of all propagated individuals. Inspection and cleaning must also be carried out after heavy rainfall (floods are a source of large amounts of alluvia) and in winter with regard to the effects of ice phenomena (e.g., the formation of frazil ice or drifting ice).

Mesh cages are usually used to perform IN-situ bioindication tests, and their cleaning is thus determined by the test conditions. Cleaning frequency varies depending on the location and season,

Fig. 66. Buddensiek bioindication plate installed on site with data logger (HOBO-Pendant, Onset) recording continually in one hour step temperature and light.

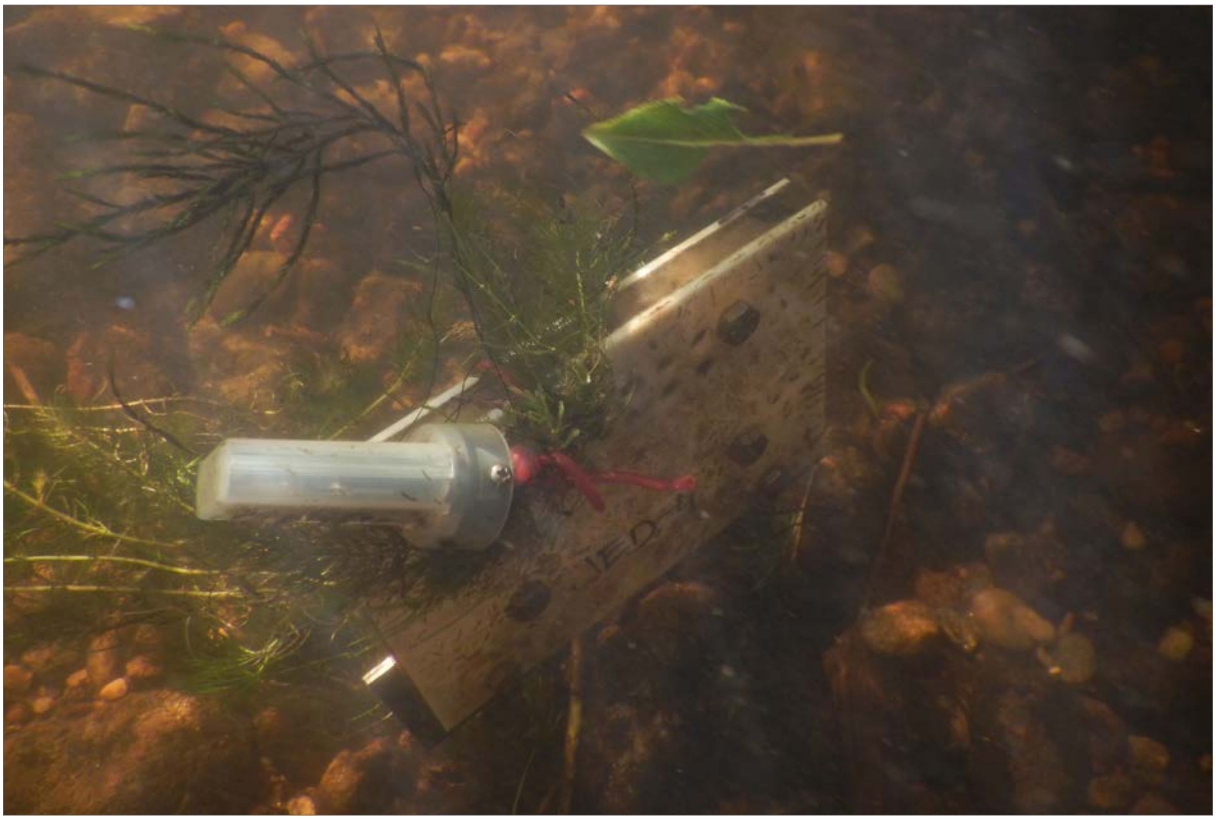

Fig. 67. Long-term installed bioindication plate with captured fragments of macrophyte vegetation and periphyton cover before regular cleaning.

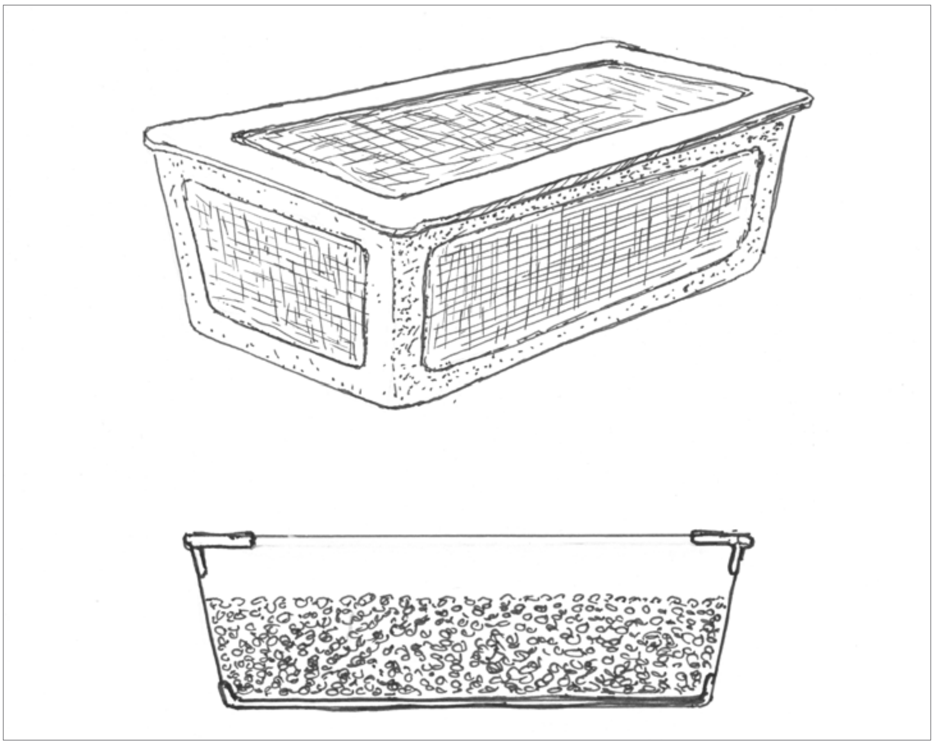

Fig. 68. Hruška sand cage is made from a plastic food container with windows covered with nylon mesh (above), and filled with washed and sieved coarse sand fraction 1–2 mm (below). The windows, as big as possible, are very important for oxygen depletion prevention – see Bílý et al. (2018) for technical drawing. Illustration: Michal Bílý.

but should be done at least biweekly. Fine alluvial sediments must be regularly brushed out of the breeding cages throughout exposure time; under normal circumstances, at least biweekly or after flood flow with increased sediment. Without cleaning the experiment measure biofilm activity and mesh clothing rate not whole river conditions. When checking the sand cage, the surface of the mesh fabric is first cleaned with a soft brush and the fine sediment is stirred and washed out by circular movements. Everything must be done under water.

7.2.1.3 Determining juvenile shell length

Juvenile shell lengths can be determined in two ways. The basic method is to photograph individuals or entire groups with a trinocular stereo microscope (**Fig. 70**). A standardized calibration grid with known mesh spacing is used to calibrate the photographs. The grid was photographed repeatedly in each series of photographs. Using suitable software, the size of juveniles is then determined with the help of image analysis; for example, Image J is available free of charge (https://imagej.net/ij/index.html). It is advisable to take at least two photographs of each individual with retracted foot and a sharp shell edge **(Fig. 71** vs. **72**).

The first step in the program is to calibrate the photo with an object of exactly known size. For this purpose, a calibration grid is compared with the FPM and thus their size is determined. It is also important to record a number indicating the magnification of the object in the microscope. When using a computer program, it is advisable to record the size of mussels by measuring the longest length between the vertices of the shell (it is usually the first chance to measure the length between the shell vertices; if the shell shape is not very regular, the measurement is repeated several times and the largest value is recorded). High measurement accuracy is required because of short exposure times (tens of µm). Another measurement option is to use the eyepiece scale of a binocular stereo microscope **(Fig. 73)**. This way, the size data is obtained immediately, but less accurately and without data recording.

In the event of an error in photograph registration, or mistaken identity during juvenile pearl mussels handling, the value in issue can usually be verified with the help of individual identification based on unique distinctive shell features (spots), if the same side of the juvenile is photographed.

Output measurements and return of individuals either to a breeding facility for further breeding or free release into a river with proven bioindicative survival – it is always necessary to observe the local affiliation of the population to the same conservation unit (Simon et al. 2015). For step by step exact work procedure see Bílý et al. (2018).

Fig. 69. Installed Hruška sand cage weighed down with stones to prevent the box being washed away by the current. Alluvia and detritus gradually settle on the box, therefore regular cleaning is necessary (Figure 57 shows a clean box just after installation).

7.2.1.4 Experiment design

Experiment design has to match the bioindication aim. It is always necessary to use a sufficient number of replications with some allowance granted (due to possible high individual mortality, e.g., due to plate clogging, fungal or bacterial infection, and plate loss too). If we assume low mortality, we can choose a plate with stocked 6 individuals (statistical minimum). In the case of expected high mortality, we can stock the plate with 12 individuals (e.g., when exposing juveniles of age cohort 0+). The number of bioindication plates at one locality should be defined according to assumed micro-habitat variability so that the results can be statistically evaluated. We always install at least 6 plates in the hyporheal due to increased mortality of individuals in the plates if the oxygen concentration at the respective site decreases. It is advisable to install the plates far enough apart so that you cannot see from one plate to another. This reduces the risk that a vandal or a curious child might destroy all installations at a given locality (in the event of good water transparency).

When stocking juvenile FPM in bulk, 50–100 individuals is appropriate. This number also depends on the expected mortality. Individuals of age cohort 2+ and older already have relatively lower natural mortality than individuals aged 1+ or 0+.

As a standard, water temperature is continuously measured during bioindication, together with the light. A data logger with continuous data recording is used for this procedure (e.g., HOBO Pendant Temp/Light, 64K). It is suitable to place the continuous thermometers directly on the surface or inside the bioindication system, especially on the stream bed or in the hyporheal with multiple replications. To evaluate the water chemistry of the given profile and its changes, it is necessary to regularly measure other selected parameters; water conductivity, the concentration of dissolved oxygen, pH, and so

Fig. 70. Trinocular stereomicroscope equipped with a camera to take photographs of juvenile FPM.

Fig. 71. Good quality photo of a juvenile with clearly visible shell edges; yellow line indicates longest length between vertices of the shell.

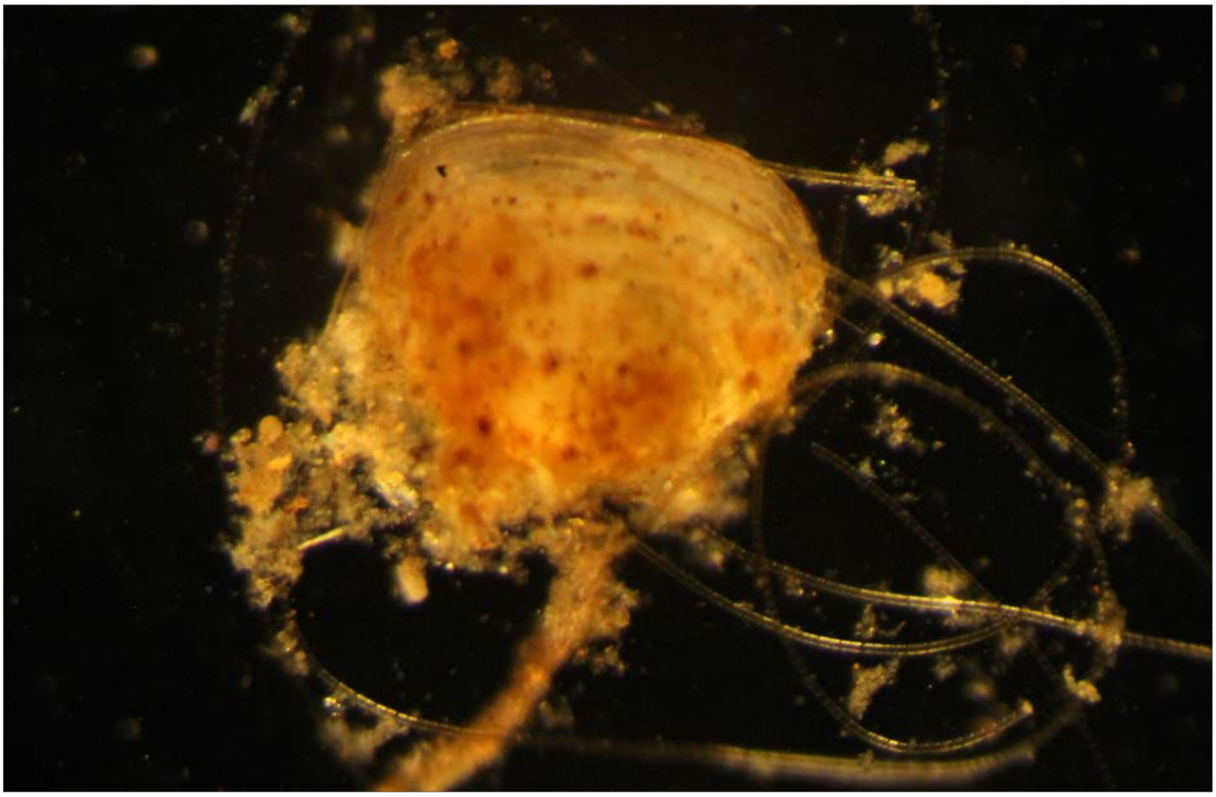

Fig. 72. Juvenile covered by biofilm and filament algae – it is not possible to be determine vertices of the shell.

on are usually measured. Data providing a basic analysis of water quality are also important. However, **one sample analysis cannot be used as proof of the overall chemistry** of a locality. This is especially true for factors that fluctuate sharply in space and time (ammonia, suspended solids).

Short-term accidents related to the stream can lead to the death of individuals in a bioindication test without reference to monitored goals (Černá et al. 2017). Therefore, it is recommended to measure selected parameters (e.g., water conductivity) continuously and, if there is an accident, to get immediate information about the current state of chemistry in the monitored stream. Telemetry stations communicating via a GSM signal are used for this purpose (e.g., Fiedler & Mágr brands in Czech Republic).

The localization of bioindication profiles is based on the basic goal of monitoring. We always choose suitable localities to compare with synchronous installation because interannual variability is important (Černá et al. 2017). In particular, it is necessary to take into account temperature conditions, water chemistry, the presence of food particles (detritus), water turbidity, and so on.

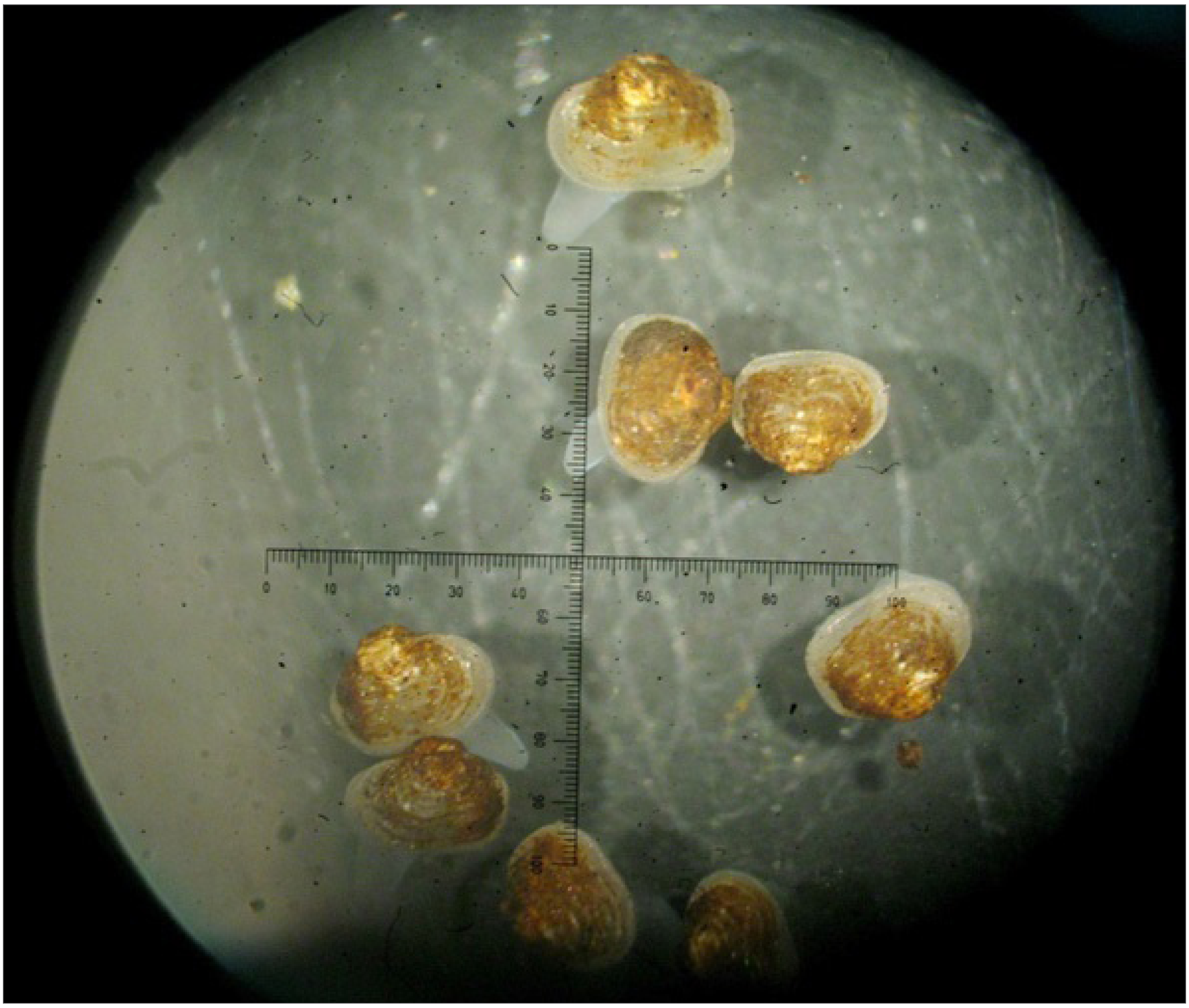

Fig. 73. Low magnification of juveniles with a binocular stereo microscope – the eyepiece scale is in the centre of the visual field.

7.2.1.5 Result evaluation and interpretation

Individual exposure of individuals in experiments allows several methods of data selection for evaluation, in contrast to older methods with group exposure of individuals. It is possible to evaluate not only mean values of the whole data set (average, median with variance, boxplots) but also groups of individuals according to their initial size. Less variable results are obtained from the evaluation of a group of fast growing individuals (e.g., the ten largest individuals in each large system '10MAX' – **Fig. 81**, or the three largest individuals of the plate of six '3MAX' – **Fig. 80**), which eliminates the effect of unpromising juveniles with delayed growth (Černá et al. 2017).

During statistical data processing, it is not appropriate to exclude extreme values connected with maximum juvenile growth in a cage or plate. It is probable that the fastest growing individuals of all stocked juveniles become the founders of a new population (J. Hruška, pers. comm.). In contrast, zero-growth survivors often occur in a large percentage of datasets, especially when speaking about exposure up to one month; it is probably a distinctive feature or disease of the individual. The data are usually highly variable even when using a homogeneous input group and stabile temperature (**Fig. 74**). This is probably a natural variability in bivalve mollusc growth (Larson et al. 2014) which can be reduced by using individuals aged 1+ or selected only fast-growing individuals to experiments. In addition to individual variability, the data set is usually affected by a particular cage or plate showing, for example, high or absolute mortality and low growth. This can be explained both by the influence of microhabitat and, for example, by infection or predation spread throughout the system. Cases of mass deaths of hundreds of 0+ or 1+ juveniles within a few days (probably due to bacterial infection), as well as chronic and acute fungal infections, have been encountered at rearing features and are also expected in rivers.

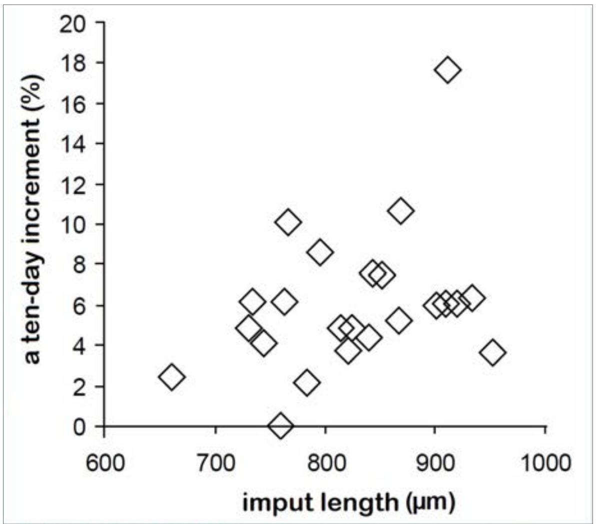

Fig. 74. Variability in the growth of juvenile FPM of 1+ cohort at a constant temperature in a laboratory. Laboratory bioindications result using detritus from Teplá Vltava River the increment varies from 0.1 to 17.6 % (N = 23), regardless of initial length.

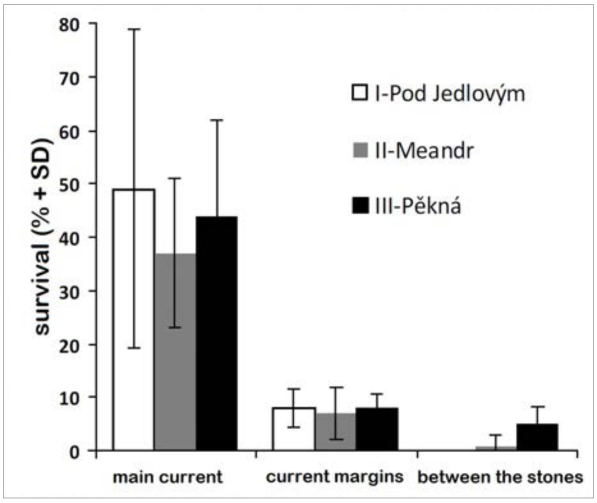

Fig. 75. Differences in mortality of individuals bred in Hruška sand cages installed above the stream bed in different positions to the thalweg, from three main sites (downstream: I–III) on the longitudinal profile of the Teplá Vltava River. Results for 2014 and 2015 cumulatively, number of repetitions: N = 30 cages installed in the main current; N = 11 cages installed in current margins; N = 6 cages installed between stones in low current.

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In the stream, we always try to install individual replications within conditions with a distinctive low variability of the microhabitat so that replications provide comparable outputs. For example, we place cages in open water in the thalweg or on its edge, at an angle of 45° to the thalweg. **Figure 75** shows that middle survival values after three months of exposure differ fundamentally when boxes are placed in a current, in vortices behind stones, or in gaps between stones but in the same time, variability inside groups is very high.

Differences in mortality and growth should therefore be carefully assessed in the context of other data from the same year or catchment (Černá et al. 2017). Very low mortality may often be caused by very low water temperatures that prevent individuals from growing. At other times, relatively low mortality may prove a favourable site within the compared localities. High mortality may be caused by several factors, such as oxygen decreases, high ammonium ion concentrations, presence of toxic xenobiotics (e.g., Roundup herbicide), or mechanical clogging of chambers. However, very high or 100 % mortality of the whole bioindication system can also be caused by infections. Therefore, more cages or plates with fewer individuals (e.g., six plates with six individuals each) should be used rather than one cage with 36 individuals.

It is also difficult to determine the causative factor of observed growth rates. Rapid growth accompanied by high mortality may indicate, for example, a eutrophic habitat where no parameter exceeded critical values at the time of the test. Favourable localities in the Czech Republic with FPM feature rather medium growth and low mortality rates. Slow to zero growth is usually caused by low water temperatures in our country (**Fig. 80** – cold location Olšinka stream). Relatively low growth rates can be caused by, among others, low-quality detritus, high concentrations of clays in water, or pH fluctuations. However, equally low growth rates can be also caused by improper cage or box installation. Therefore, it is always necessary to use more than one system in parallel, like Buddenisiek plates and Hruška sand cages in Vltava River (Černá et al. 2017). Repeated bioindication for several years in a row gives well-proven results (comparison of **Fig. 76** and **Fig. 79** shows similar relative differences in growth for 1+ cohort).

7.2.2 In situ bioindications options

7.2.2.1 Mesh cages or sand boxes?

In order to obtain adequate data, the choice of bioindication system is a crucial decision. Buddensiek plates are a traditional, low-cost, and relatively widely used option. Their advantage is the possibility of individual exposure and individual evaluation. Large breeding chambers also allow using small groups of younger juveniles. However, these plates also impose a whole range of limits. Juveniles are kept out of the hyporheal, so there is no risk of lack of oxygen. At the same time, however, they are directly exposed to bad conditions (extreme floods/droughts, poisoning, etc.). The fact that small mussels are deprived of the possibility to move between the sediment grains and thus choose the optimal position can also be a drawback. At some localities, individual chamber cages are also colonized by a large number of benthic organisms (*Chironomidae*, *Plecoptera* – stoneflies, caddisflies) that penetrate the mesh as the first instar larvae; in the following instar, they may act as predators or block the chamber with their fibres. Rearing boxes filled with sand (whether Hruška sand cages or the newly introduced stainless steel mesh cylinders) comply more with the natural juveniles' microhabitat but they do not allow individual data evaluation (Bílý et al. 2018). Traceability of juveniles, especially of lower age cohorts, is also more difficult in this system. The use of sand boxes for bioindication places great demands on a sufficient number of juveniles (within the CAP FPM, there usually are 100 individuals 1+ per box). It should be noted that if the tests are performed under relatively optimal conditions (adequate growth, medium or low mortality), they can also be considered as young individuals' breeding. These individuals can be used further to strengthen populations or bioindications in subsequent years.

In the comparative experiments performed so far, cages and boxes (even when installed in the same flow and the same microhabitat) have produced different results (**Fig. 76**). In boxes, juveniles aged 1+ grow faster, but have significantly higher mortality, as reported by Černá et al. (2018). These facts will be the subject of further research.

7.2.2.2 Under the stream bed, above the stream bed, or in open water?

Another crucial decision when choosing experimental design concerns the position of bioindication systems in relation to the stream bed. So far, all published works except Bílý et al. 2020 and Černá et al. 2017 works have been based on exposure in open water above the stream bed. This kind of installation is labour-saving – systems are easily cleaned and inspected. However, placement above the stream bed eliminates the influence of several factors that are thought to be limiting for the growth

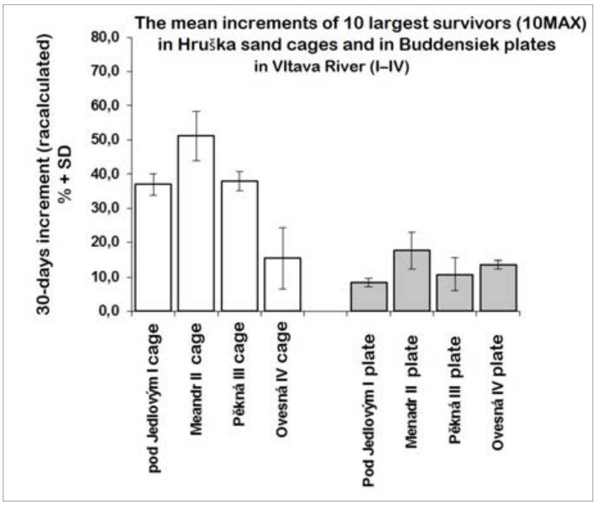

Fig. 76. Methodical comparison of juvenile FPM growth in Hruška sand cages (exposure 3 months, mortality 71–92 %, position above the stream bed) and in Buddensiek plates (exposure 1 month, mortality 3–9 %, free water position) installed at the same sites being converted to one-month exposure in 2014. The 10 largest survivor increments (10MAX) were selected to eliminate the effect of juveniles with delayed growth and faster-dying individuals. At each site, there were 6 mesh cages of with 6 individuals per cage and 4 sand boxes of with 100 individuals per a box. The average number of surviving individuals, from which the 10 largest were selected, was 21 in sand boxes and 33 individuals in mesh cages due to significantly high mortality rate in sand boxes – more details in Černá et al. (2018).

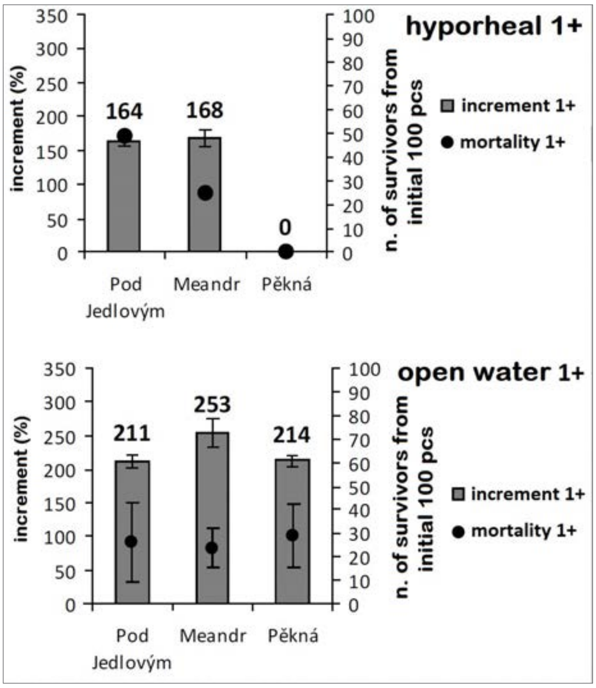

Fig. 77. Comparison of FPM growth and survival in hyporheal (picture above) and open water (picture below) in Hruška sand cages at 3 main profiles of the Teplá Vltava River, using individuals aged 1+ and 3-month exposure in 2014.

and survival of juvenile FPM in the hyporheal. Good survival and growth of juveniles exposed in open water can thus be achieved, for example, in heavily eutrophicated localities, especially during short-term exposures.

If the systems are installed at the stream bed level, the conditions correspond more to the natural habitat and the supply of food particles drifting along the stream bed is available. However, there may be a problem with oxygen supply due to faster clogging of the mesh. The Buddensiek plate in a fixed position and depth makes it impossible for juveniles to choose the optimal position in the environment and it thus becomes unnatural and risky. If the systems were installed under the stream bed (Jandáková et al. 2015, Pasco et al. 2015, Černá et al. 2017), the most natural conditions would be achieved. **Figure 77** shows a comparison of bioindication results concerning above and below stream bed cage installations.

The appropriate depth and exact location must be verified with previous measurement of the oxygen concentration (Černá et al. 2017) or redox potential at the stream bed. The oxygen content must be further monitored during the experiment (preferably using a continuous probe) as oxygen ratios can change rapidly in the hyporheal, depending on the environment.

The lack of data concerning FPM juveniles under the stream bed raises many questions. At what depth should be juveniles exposed? Should upwelling or downwelling parts of stream beds be chosen? What is the optimal grain size of the stream bed substrate? Scientists around the world have been searching for answers to these questions. The most suitable is probably a shallow hyporheal to 10 cm deep, a soft gravel or sand gravel stream bed with good oxygen supply that does not differ much from the surface water (Geist & Auerswald 2007, Černá et al. 2017). Preliminary ethological study from Vltava River showed, that 1+ old juvenile FPM preferred position 3 cm below the river bed (Bílý et al. 2020).

7.2.2.3 Exposure time and season

Previous published works were based on a long exposure period, usually lasting the entire vegetation season (three or more months, at least from June to August). During such a long exposure, the impact of the main variables on a given locality is significant. However, the disadvantage of such a long exposure is the higher risk of losing a cage and a longer time for its necessary maintenance on site. Some authors use much longer exposure time, such as eight months (Bílý et al. 2020) nine months (Denic et al. 2015), fourteen months (Pasco et al. 2015) or even more (a summary is provided by Gum et al. 2011). In contrast, shortened exposure time was tested by Douda et al. (2015) who evaluated an exposure time of one month as sufficient (to detect differences in growth) by comparing a large series of plates with three replications for a significant temperature gradient. The big advantages are significantly lower mortality of juveniles and reduced labour intensity. However, due to the greater influence of temperature fluctuations and local precipitation during part of the summer season, the short exposure time seems to be disadvantageous (Bílý et al. 2018). Shorter exposure times are especially common for testing potential toxic effects connected with construction work carried out in catchments with FPM in the Czech Republic, as prescribed by the relevant conservation programmes (AOPK ČR 2013a)

7.2.2.4 Age and size of juveniles

In contrast to authors from abroad, bioindications are used in the Czech Republic with individuals reaching the end of the first growth period (1+ cohort with an individual size of 800–1,100 µm). The reason is their significantly lower natural annual mortality compared to the younger 0+ cohort. As indicated by preliminary tests (Hruška 1999), in the first year of breeding there is always significant mortality of bred individuals due to the elimination of non-perspective individuals from the growing group (for example in the Blanice River population fed with detritus from springs). If natural selection occurs during bioindication tests (genetic or parasitic phase-dependent), the results may be significantly distorted (see **Chapter 6** for more details).

Individuals of the first grow cohort 0+ are suitable if we want to test whether conditions at the site allow natural reproduction. This is because 0+ is considered the most vulnerable age period. Another reason for using this test is a comparison with non-Czech studies, which usually do not have older individuals available in sufficient numbers. A comparison of bioindications of individuals in five

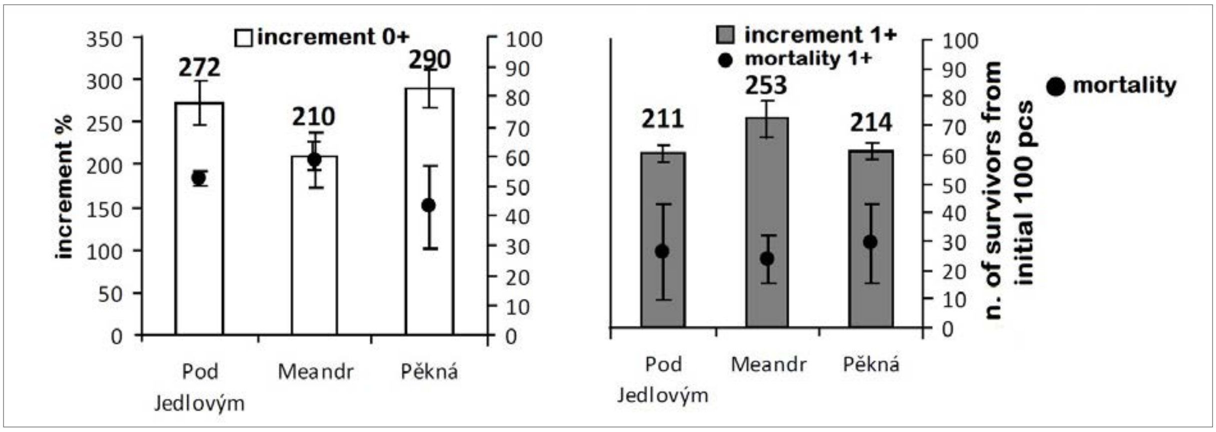

Fig. 78. Comparison of FPM growth and survival in open water in Hruška sand boxes in 3 main profiles of the Teplá Vltava River using individuals aged 0+ (white bars) and 1+ (grey bars) (± SD), 3-month exposure in 2014.

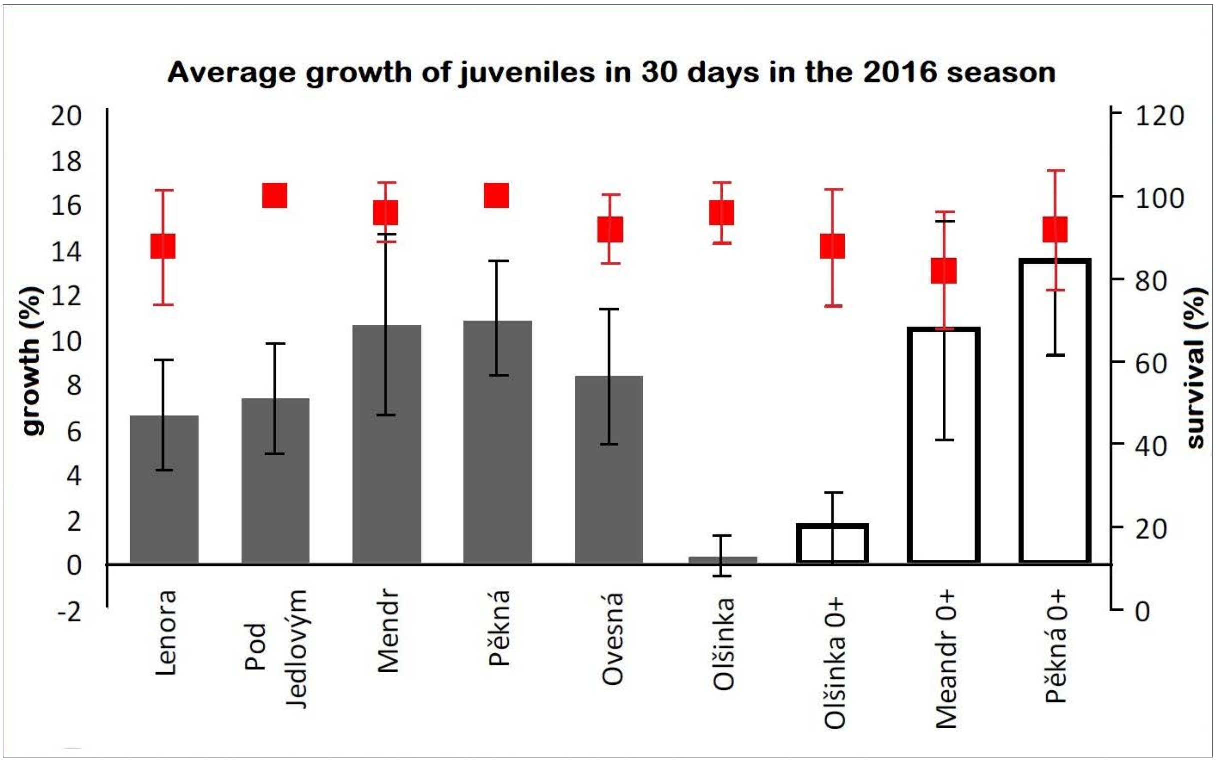

Fig. 79. Comparison of individuals aged 0+ (white bars) and 1+ (grey bars) used for 1-month exposure in Buddensiek plates in free water position. Red – mortality (± SD).

age cohorts is shown in **Figure 34** in **Chapter 5** (age cohort 0+ shows almost 100 % mortality and minimal growth in one of the monitored streams).

To get a comprehensive view of growth and survival at a given locality, it is recommended to use multiple age cohorts in parallel. **Figures 78** and **79** shows an example of test results where plates and sand cages were used. This particular case reveals that the mortality rate in sand cages differs (**Fig. 78**). As for plates mesh cages (**Fig. 79**), the differences found in the monthly exposure in 2016 are very small (juvenile age cohorts 0+ show higher mortality and overall greater results variability).

Grow rate is absolutely and relatively dependent on the initial size of individuals, when larger individuals grow faster – see **Chapter 7.2.1.6**. In contrast, growth-lagging individuals can survive for several years (especially in cold conditions) with only minimal increment before they die (J. Hruška and B. Dort, pers. comm.). The share of individuals with lagged growth within the mass-evaluated sample then significantly decreases the grow rate in all exposed microhabitats. This can be prevented by using older generations with already deceased lagged-growth individuals, at least in age cohort 1+ or older. Therefore, groups of individuals with a comparable average initial size should always be included in collective experiments. For this reason, it is suitable to take at least a group photograph of all individuals included in each experiment (**Fig. 81–83**).

There might be a problem with initial size if we perform one bioindication test in June and another one in August (e.g., to evaluate the immediate effect of management intervention). The initial size of individuals of the same age cohort in August should always be larger, which makes comparisons difficult.

7.2.2.5 Collective or individual exposure in bioindication facilities?

If the individuals are bread individually in mesh cages, then it is possible to evaluate the results concerning the factors of respective individual, cage, and locality. However, from Czech experience, growth in mesh cages is usually smaller in comparison to sand boxes (Černá et al. 2017). It cannot be discounted that the mesh cage environment is too simple for juveniles.

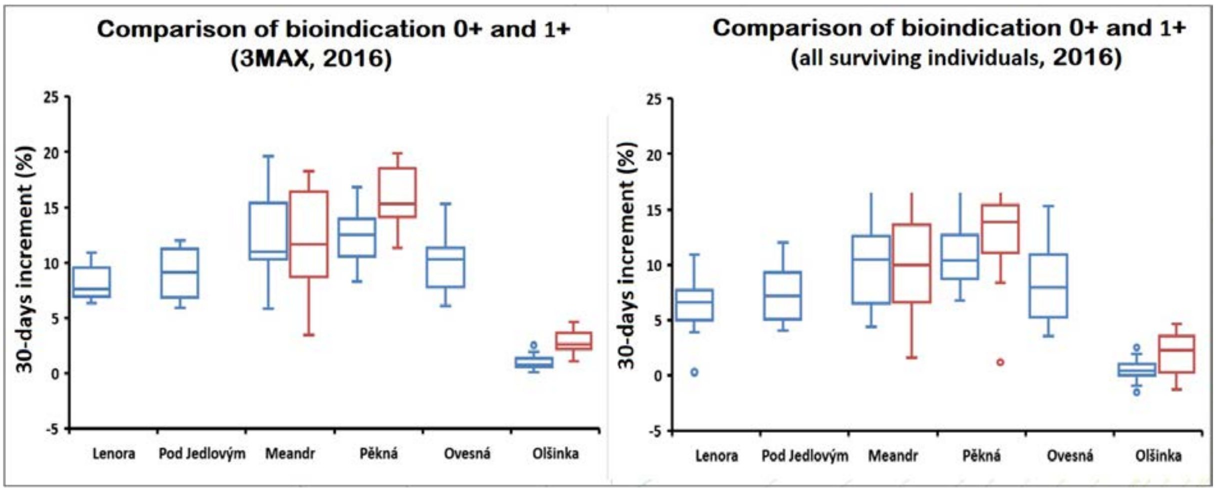

Fig. 80. Comparison of average size of all surviving individuals (right graph) and 3 best growing individuals (left graph) for boxplots (red cohort 0+, blue cohort 1+). The use of the 3 largest individuals of the 1+ cohort only slightly increased the average obtained and reduced the variability. For individuals of 0+ cohort, this effect was more significant (as a result of higher mortality). Therefore, it is more appropriate to use older individuals (1+).

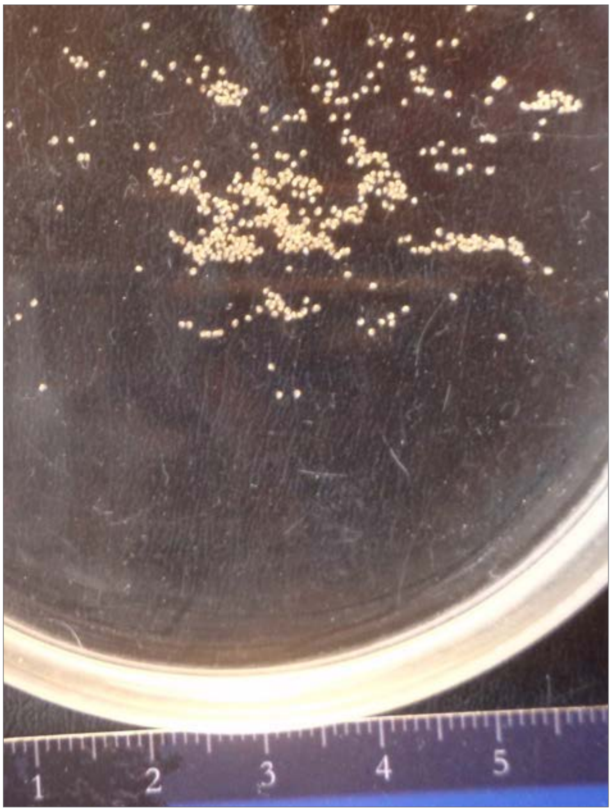

Fig. 81. Actual size at beginning of the experiment (100 individuals). Size measurement in a group of individuals intended for an experiment. Photographs taken at about 20× magnification enable measurement of individuals in the selected group. It is recommended to measure the 10 largest individuals (start measurement of large individuals and then select the 10 largest). It is also possible to select a random sample; however, to measure all individuals it would be necessary to use photographs where there are no overlaps, significantly increasing the workload.

Individuals bred in large groups can be evaluated only as a summary of the whole system (sand cage evaluation). Another possibility is to evaluate a certain number of fastest-growing individuals – 10MAX characteristic. However, it is necessary to remember whether 10 % of the largest individuals have been selected or, for example, 50 % – the choice will fundamentally affect the final absolute value and variability too (Bílý et al. 2018).

Hypothetically, it is possible to use mass photography to identify some individuals according to the characteristic spots on the shell and then to evaluate them individually. As for sand cages, ensuring a sufficient number of repetitions is usually difficult, but an easy and standard condition when using cages.

7.3 Laboratory bioindications (EX-situ)

7.3.1 Methods and procedures

Laboratory (EX-situ) bioindications are less common than IN-situ exposures. Procedures within the CAP FPM involve regular evaluation of food particles taken from the respective sites, mainly to eliminate the effect of temperature in the natural environment. Furthermore, it is possible to test various types of artificial or modified feed used for rearing (see **Chapters 5** and **6**), detritus decomposition during its transport by river networks (Slavík et al. 2016), and many other parameters. These bioindications are based on the assumption that growth of juveniles during a test is influenced mainly by supplied particles at a constant temperature.

The standard Hruška methodology presented by the CAP FPM assumes a twenty-day test at 19 °C, carried out in a 500 ml plastic container. In the middle-period of the experiment, old detritus is replaced with a fresh. Ten mussels of the 1+ cohort are kept in the container.

Control experiments without provided food were also used to verify whether juveniles were able to grow even without added feed using their own energy reserves, biofilm, or solutes (**Fig. 32** and **33** in **Chapter 5**).

7.3.2 Detritus sampling

A fundamental issue for testing detritus carrying capacity in laboratory conditions is detritus sampling. The layman thinks of detritus as „sludge“ or „dirt“ from the stream bed. In fact, detritus is a very complex, spatially structured, flocculating material composed of semi-decomposed plant remains, a diverse range of single-celled „protozoa“, algae and bacteria, mineral particles of fine sand, clay flakes, and precipitated colloidal substances. **Chapters 5** and **6** discuss detritus composition and structure in more detail.

Detritus sampling for bioindication evaluation must be performed with great care. Detritus collected from the surface oxic layer in springs may have excellent carrying capacity, but the mass found just a centimetre deeper may contain an anoxic „rotten“ material without any carrying capacity.

Three basic types of detritus can be distinguished according to the test aim: deposited, floating, and hyporheal. These types of detritus found at the same locality differ in composition and lead to different results in bioindication tests (**Fig. 28**). Sampling methods of these types of detritus are described below.

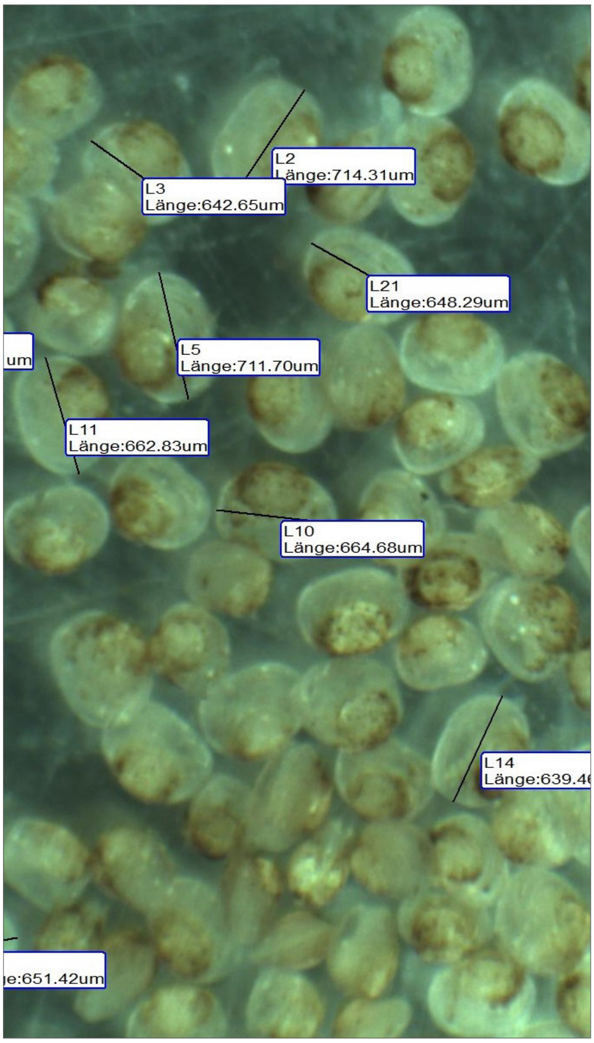

Fig. 82. Measurement at beginning of the experiment – size about 0.65 mm.

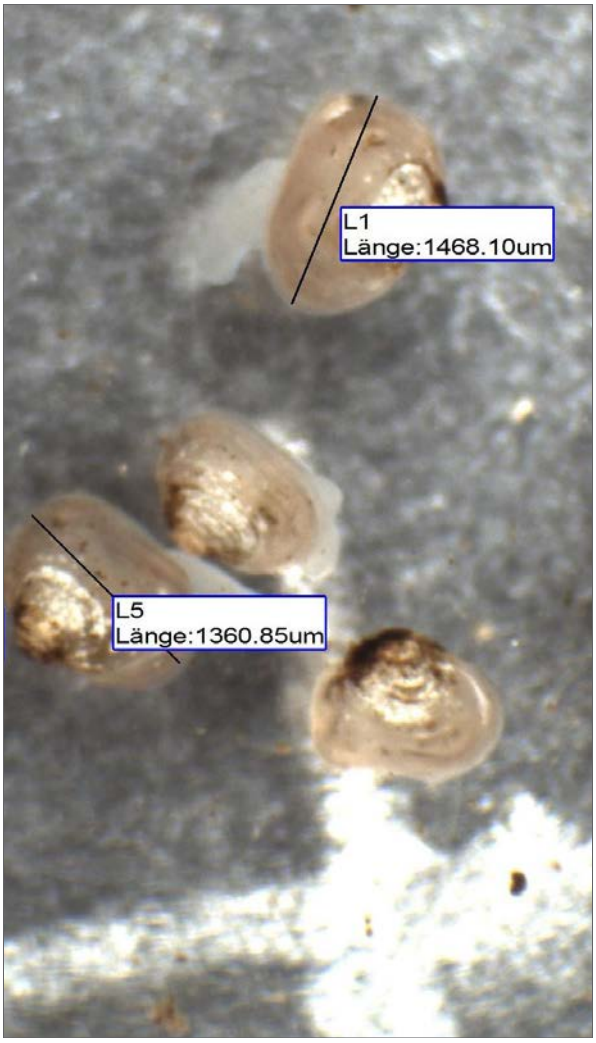

Fig. 83. Measurement at end of the experiment (31 individuals) – size about 1.2–1.4 mm.

7.3.2.1 Deposited detritus

Deposited detritus means fine deposits on the stream bed or in spring pools or outflow trickles (see **Annex 5** or Tichá et al. 2012) . It is a light material that may be brown, dark grey, or black and resembles activated sludge from wastewater treatment plants (**Fig. 90**). This detritus is found in layers with sufficient oxygen saturation and is easily resuspended by the stream and stored again. After a more intense flow rate, it can be difficult to find places with deposited detritus in a stream. In contrast, in springs with a constant flow, which is defined by the force of the spring source, its occurrence in places sufficiently far from the fast current or concentrated spring outflow is constant (**Fig. 84**).

Detritus should be sampled in slow-running waters or shallow microhabitats (e.g., springs or spring streams). A ladle or shallow container should be immersed so that the lower layers (with a different oxygen regime) are not agitated. In deeper or fast running streams, detritus is taken with a large syringe.

It is possible to allow the detritus to be concentrated by sedimentation in larger vessels, but a sufficient proportion of water must always be preserved (volume ca. 1:10) to maintain sufficient oxygen concentration. In practice, the character of detritus can also be taste-tested, if the detritus is taken from localities with drinking water (rotten material can be easily distinguished).

Fig. 84. Deposited coarse detritus with a particle size of about 1 mm (red arrow) and deposited fine detritus in flakes (yellow arrow) in a spring source. The detritus is sorted by size by the strength of the current. Under normal conditions, a mixture of size components is visible.

Prior to testing, the detritus is strained through a 40 µm sieve to obtain a test-size fraction corresponding to the particles ingested by mussels and to exclude larger benthic animals (macroinvertebrates + meiobenthos). Use of another mesh size is possible, but morphological and chemical composition of different size fraction is different as well (Tichá et al. 2020). Hydrobiological standard for FPOM (fine particulate organic matter) is mesh size 1000 µm (Lamberti & Hauer 2017).

7.3.2.2 Drifting detritus

Detritus deposited on the stream bed is usually formed by sedimentation of detritus dispersed in open water. Adult FPM cannot feed on this type of detritus unless it is resuspended by a larger flow or animal activity. Drifting detritus is more important for the mussel nutrition. However, its concentrations are usually very low in natural habitats (baseline flow rate around 2 mg/l). To obtain this type of detritus, sedimentation of material obtained from a large volume of water is necessary. A Coshocton sampler and a set of sedimentation barrels can be used to perform representative sampling in small streams (allowing particles larger than 1 mm to be sampled; Cuffney & Wallace 1988, Wallace et al. 1991, 2007) or a gravity-type sampler providing a continuous sample flow (DDG) (**Fig. 85**, Simon & Fricová 2009, Tichá et al. 2020). Quantitative sampling performed in larger streams is expensive and requires special samplers. A representative composite sample can be obtained using a pressure-type sampler (DDP) (Patent CZ 303836). Both the DDG sampler and the DDP sampler only take material with a particle size less than 1 mm. Using nets for sampling is not suitable for the given application because the finest fractions representing the feed of FPM penetrate nets uncontrollably until colmatization.

To perform the tests, weekly exposure to a DDP or DDG sampler is sufficient under normal conditions. A similar increase in juveniles was found in bioindicative comparisons of a series of samples of deposited and drifting detritus at the same localities (**Fig. 28**). From a chemical point of view, these kinds of detritus differ (e.g., content of mineral particles), which is more significant on the stream bed (**Fig. 25**, **Chapter 5.4**).

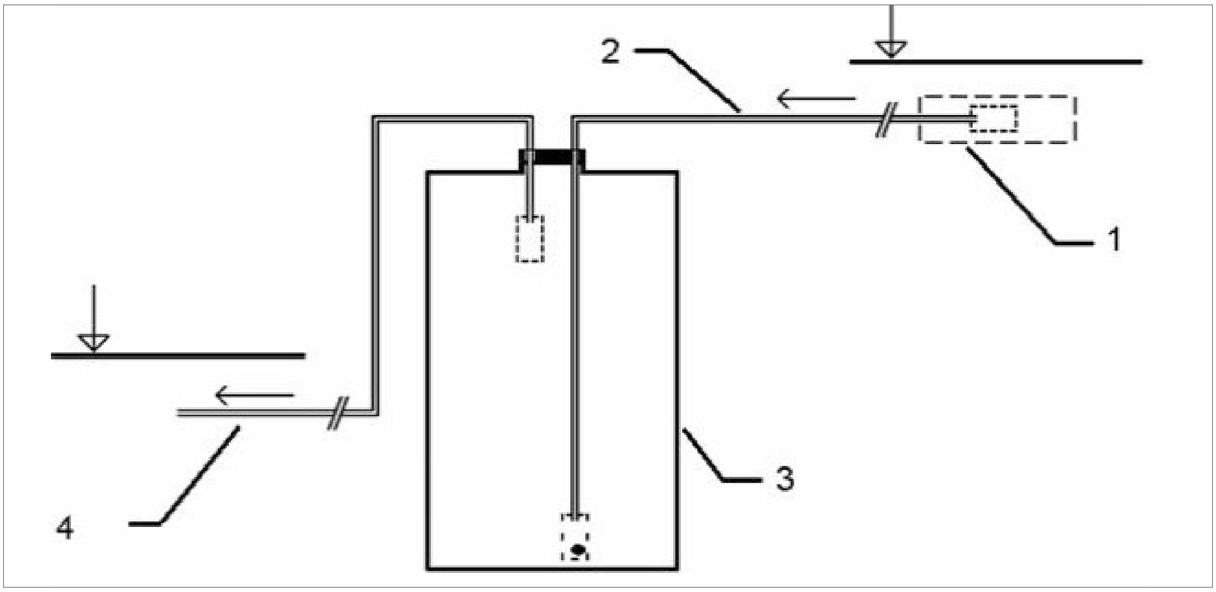

Fig. 85. DDG sampler scheme. 1 – suction basket placed in the stream, 2 – flexible supply hose, 3 – body with sedimentation space (5-litre PET bottle), 4 – flexible drain hose; the upper level shows the location of the suction basket in the upper part of the stream and the lower level of the mouth of the waste pipe in the lower part of the stream with a large slope.

7.3.2.3 Hyporheal detritus

Detritus in the hyporheal in the interstitial spaces between stream bed sediment grains can originate from multiple sources. In places of downwelling, it is a material that was carried or floated by water some time ago. Under the stream bed, the detritus can also be formed by the release of microbial gain on the sediment grain surface. Under specific conditions, it can also be brought by underground flow from the surrounding area (see **Chapter 5**).

When taking detritus from river and stream hyporheic zones, a penetrating hollow probe is used, driven to the required depth. The oxygen concentration is checked in the first volume of water sucked in with detritus. It is not logical to test detritus from places with oxygen deficiency. Detritus taken from areas with oxic hyporheal is gradually aspirated and allowed to settle in bottles. Collection is time-consuming. While performing a comparative experiment, juveniles of all localities fed with this type of detritus proved only minimal growth (**Fig. 28**). A greater proportion of mineral particles was also found in hyporheal detritus.

7.3.3 Laboratory bioindications in containers (after Hruška)

The basic method of bioindication performed in laboratory conditions was described by Hruška (unpublished, more recently summarized in the CAP FPM, AOPK ČR 2013a). Five juveniles photographed together are placed in a 400 ml food container in a bed area of 220 cm^2. There is a 1–2 cm layer of water in the container and a layer of detritus sieved with a 40 µm mesh. Its height is 1–2 mm. To perform the bioindication test, FPM are kept at a constant 19 °C. After ten days, the detritus is replaced with a second batch of the same detritus sample (stored at 4 °C). At the end of the test, the mussels are separated from the detritus on a 0.5 mm sieve and measured again. The average size or maximum size reached in the container is used as a growth indicator.

This test is primarily intended to determine growth, not mortality. If material toxicity is suspected, it is recommended to exclude toxic samples in advance. A simple short-term test can be performed using sensitive but unprotected local benthic organisms, such as mountain *Crenobia alpina* (flatworm). The advantage of the container test is a safe environment for juveniles, which can be buried in detritus and possibly apply gathering behaviour (**Fig. 53**). Large amounts of water and detritus per individual should eliminate any problems caused by metabolic products. The disadvantage of this method is the lack of information concerning the increase of individual mussels (the 'individual effect'). The method is also demanding because a sufficient amount of detritus must be obtained. To perform enough repetitions of the test, it is also necessary to have a large space with a constant temperature. Therefore, comparative tests were performed to develop a suitable micromethod that would work with a smaller number of individuals and smaller volumes of water and detritus tested (**Fig. 86, 87**).

7.3.4 Micromethod of detritus evaluation by FPM 1+

The micromethod is based on individual exposure of juveniles in a small breeding container. Petri dishes and plastic test tubes were compared with the classic method in containers developed by Hruška.

In both test tubes and Petri dishes (**Fig. 89**), there was 25 ml of water per individual and 1.5 ml of pipetted sedimented detritus (**Fig. 90**). The test tubes were cultured in an oblique position to form

Fig. 86. Breeding containers for mass breeding of 5 juveniles (method according to Hruška) and Petri dishes for individual exposure.

Fig. 87. Micromethod in test tubes (individual juvenile mussels breeds) with fewer space requirements and less need for detritus in the sample.

a layer of detritus in the lower part of the bed, under which an individual could hide or climb to the bare bed without detritus (**Fig. 88**).

The results of the comparison of the eight samples with all three methods are shown in **Figure 91**. The statistical difference of average increments was not significant; only for Petri dishes was the variability of results greater. The fact that a juvenile could not hide under detritus in a very large Petri dish could be understood as a potential stress factor. In contrast, in test tubes cultured in an oblique position, the detritus under which a juvenile mussel could hide was always accumulated. Therefore, the use of test tubes with a sufficient layer of detritus can be considered more appropriate than Petri dishes.

Control samples without detritus were also tested. In this experiment (**Fig. 92**), even very small volumes of water per mussel were tested to find the lower limit for bioindication breeding. A volume of 10 ml was beyond this limit and the growth of FPM here was significantly worse – probably due to an accumulation of metabolic products that could not be removed by microorganisms living in detritus, as described by Eybe et al. (2013). Therefore, we recommend keeping the standard minimum water volume of 25 ml per individual 1+ cohort to perform short-term tests.

Fig. 88. Micromethod of laboratory bioindications in test tubes. The large food storage box can hold up to 48 test tubes for individual exposure of juvenile mussels.

Fig. 89. Cultivation in a laboratory refridgerator at 19 °C in a comparative test of methods (top shelf – test tubes in an oblique position; lower shelves right – containers according to Hruška's method; lower shelves left – Petri dishes).

Fig. 90. Detritus for bioindications must be kept at a low temperature and samples carefully pipetted from the bottom of the container.

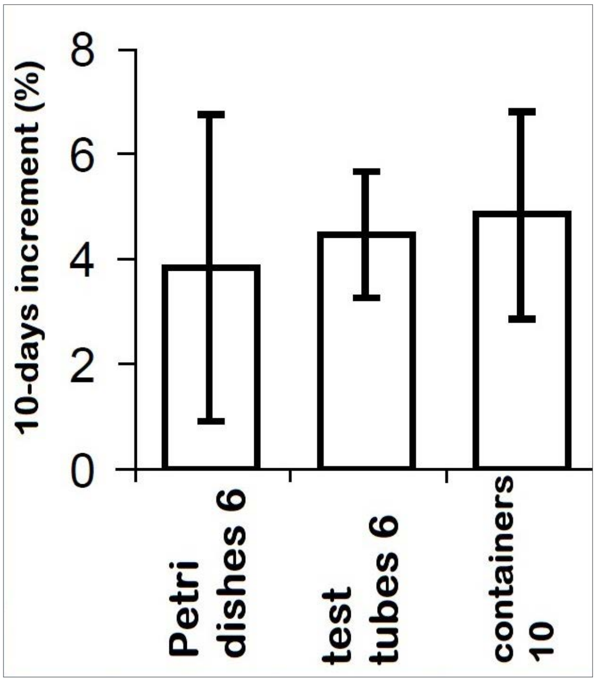

Fig. 91. Comparison of 3 methods of laboratory bioindications in terms of juvenile growth rate of 1+ cohort. The following were tested: micromethod in a Petri dish, micromethod in a test tube, and classic Hruška's method in a container holding 8 different samples of detritus from the Teplá Vltava and Blanice River catchments. For each micromethod, 6 individually exposed mussels were used; containers held 10 individuals in 2 groups of 5; a total of 48 + 48 + 80 juveniles were used.

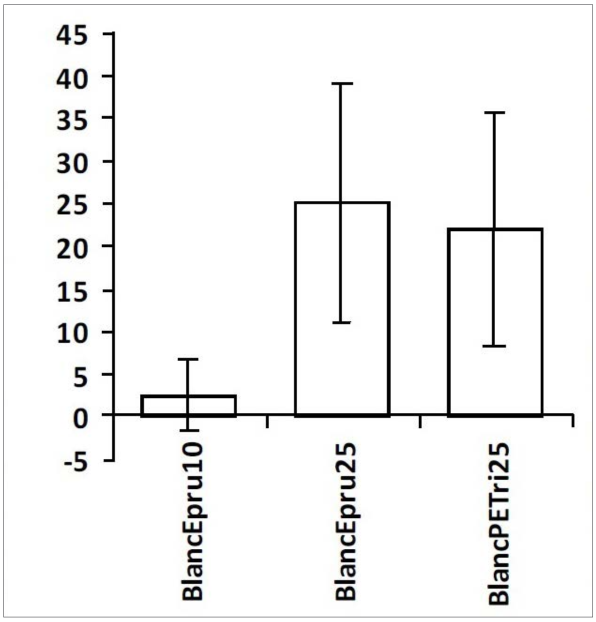

Fig. 92. Testing of minimum size of a breeding container for 1 FPM of cohort 1+. In a water volume of 10 ml, the growth of individuals was significantly lower than at 25 ml, regardless of whether in a Petri dish with a water column height of about 5 mm was used or a test tube with a water column height of 31 mm (food-free test focused on the effect of individual excretion, N = 12).

7.3.5 Summary of bioindication methods

The methodology above summarizes procedures needed to implement two basic options for bioindications for the requirements of both the CAP FPM and research.

On site bioindications (**IN-situ**) are suitable to assess the suitability of a FPM habitat and also to monitor possible toxic effects arising during various construction works in catchment. The usual standard is to always use several systems (sand cages or Buddensiek plates with individuals of 1+ age cohort) to better evaluate the results. For mesh cages with six individuals of 1+ cohort intended for one-moth bioindications, 4–6 mesh cages per site should be used.

When evaluating field data, it is sometimes possible to reduce natural variability of the results by evaluation focused on faster-growing individual increments (3MAX for the plate or 10MAX for the sand cage).The disadvantage and, at the same time, advantage (in the case of evaluation of mussel habitat) is the combined effect of many factors that are usually indistinguishable. To assess the suitability of a habitat for juveniles, the real situation is the nearest bioindication located below the stream bed. However, these still face a number of methodological problems and are very expensive.

Laboratory bioindications (**EX-situ**) cultivated at constant temperatures are especially suitable for evaluation of detritus quality or various modifications of breeding techniques. When collecting detritus, it is necessary to carefully follow the sampling methods so that only detritus in an oxic state (not rotten) is sampled. In contrast to the proven method of laboratory group bioindications performed in large plastic containers, the micromethod in test tubes with individual exposure of individuals was newly tested (at least six individuals per sample). This enables keeping individuals separately and improves the possibility of statistical result evaluation.

The main disadvantage of bioindication evaluation is the need to ensure enough juvenile mussels of the required age in advance. Installations at sites above the stream bed can be stolen or damaged by floods. While performing laboratory tests, it is necessary to allow the possibility of sudden death of juveniles due to infection. The measurement of increments using image analysis requires precise work and regular calibration of instruments and a reliable calibration grid. Photo archiving results in increased demands on the volume of archived data, but allows evaluation to be refined or modified at any future time.

Interpretation of bioindication results requires special care. It is always necessary to compare the evaluated site or sample with control bioindications performed using the same method. This is the only way that bioindicative evaluation can provide valuable information that is otherwise difficult to obtain for both the practical protection of FPM and research of the species ecology.

Chapter 8: Principles of management and use of river catchments populated with freshwater pearl mussels

Authors: Ing. Kateřina Rambousková, Ing. Věra Kladivová, Mgr. Jan Švanyga, Mgr. Ondřej Simon
Reviewers: Mgr. Tomáš Bodnár, Robert Ouředník

This chapter describes the main management principles and recommendations for the owners of the land within catchments populated by freshwater pearl mussels (FPM). Mere legislative protection and efforts by nature conservation authorities within Action Plans cannot improve the current state of the FPM population in the Czech Republic. Therefore, the main objective of this part of the methodology is to provide the public with an introduction to management of river catchments with FPM.

The authors would like to provide methodological support to land owners who often do not have enough information from nature conservation authorities on how to act with regard to catchments and land populated by FPM. In interviews or surveys, local inhabitants have repeatedly asked for clear and accessible instructions regarding which procedures pose a risk to the FPM and therefore should be avoided. For effective protection of streams with FPM, the help of people who are most familiar with this environment and who live there is crucial – this includes mayors, farmers cultivating land surrounding streams, foresters, fishermen, holiday makers, and other local inhabitants with a positive attitude towards nature.

8.1 Management principles and recommendations for forestry

There are two types of forests in the areas populated by FPM. The first are stands that are a part of **relatively large continuous forest complexes with a predominance of spruce** with sloping terrain, where small spring areas are located. The principles of forestry management in such stands are stipulated by management plans available from local forest management entities.

The second type of the forest are **small forests and stands along watercourses** with a total area of several hectares (**Fig. 93**). This type includes waterlogged sites in the floodplains of streams and large helocrene springs (**Fig. 94**) on sloping land between the stands. The composition of the sparsely distributed trees or groups of trees is dominated by *Alnus* sp. (alder) of various ages and *Salix* sp. (willow) in a shrub form, as well as *Betula* sp. (birch), *Populus* sp. (aspen), and *Acer pseudoplatanus* (sycamore) with *Picea sp.*

In the broader context of catchments with FPM and their protection zones, we can also find **forest ecosystems which are, however, not located on land intended for forest functions** – meaning woody plants growing outside of forests. These ecosystems develop as a result of natural succession in non-forest areas which were originally used as pastures.

The following subchapters provide a detailed description of how to manage the aforementioned forest types in accordance with the needs of FPM.

Fig. 93. Semi-open landscape with scattered groups of trees and riparian vegetation.

Fig. 94. An extensive helocrene spring (a non-freezing wetland spring that is fed by groundwater throughout the year) in a meadow.

8.1.1 Forest stands

In forest stands, efforts should be directed at helping the development of forest communities which are as natural as possible and regenerate naturally. Activities should always be focused on forest stands, especially their composition, additional planting, as well as the protection of plantations. Emphasis should be put on natural species composition and increasing the stability of the stand by support of promising individuals of the target tree species.

Young, predominantly coniferous stands require intensive management, resulting in the creation of sparse and irregular structures. Thinning should not be carried out schematically but with varying intensity for each group of trees, with gradually decreasing intensity away from a watercourse (very intensive interventions are suggested along watercourses). It is recommended to **preserve or create gaps in the stand** in order to lighten and warm up the primary water network or to increase the stability of spruce stands in waterlogged areas.

Wood and branches from thinnings (wood brash) should always be removed from a watercourse, as well as its banks, and taken at least 3–6 m from the watercourse and distributed evenly in the stand (creating heaps is not advised).

Mature stands should not be intentionally renewed in order to achieve maturity for harvesting but all interventions should be focused on creating sparse and irregular structures and achieving the same natural development cycle of a natural forest, as in the middle-aged forests.

FPMs benefit from partial interventions on small areas, especially around waterlogged drains and forest rills resulting from forest land development, and which are connected to the watercourse and thus to the entire catchment. Inappropriate forestry management methods are clearly summarized in **Table 1** and analyzed in detail in the following chapters, including the appropriate measures to adopt.

Quality of water drained from forest complexes

With regard to the quality of run-off water from forests, emphasis should be put on adjusting stand composition in order to reduce the areas influenced by spruce needle-fall. The strong acid reaction of run-off water (pH 5–6) deteriorates the conditions for FPM. According to the Action Plan, the lower limit for water reaction (pH) tolerated by FPM is 6.

During attempts to protect plantations (fir, hardwoods) against game, it is crucial to limit the use of chemicals in the catchments with FPM. This is related to the elimination of large-scale use of forest protection chemicals (insecticides, pesticides, arboricides). Application of chemicals, including game repellents, should always be **discussed with local conservation authorities**.

With regard to the quality of run-off water from harvested stands, wood brash should not be burned within 10 m of a watercourse and its tributaries. Large amounts of ash washed into a watercourse rapidly deteriorate the water chemistry. Spreading ash onto herbaceous vegetation is recommended because the soil under it is able to contain and use the nutrients and thus prevent their infiltration in the water environment. The further from the watercourse this measure is taken, the more effective it is.

The quality of run-off water may also deteriorate due to branches and bark blocking tributary streams during felling. This condition is often associated with a risk of development of toxic chemicals (e.g. ammonium ions, phenols, or tannins) which are subsequently washed further into the catchment. This risk particularly increases with low flow rate and rising temperature of water during the summer months. Therefore, during harvesting. or soon after, the condition of the adjacent watercourses should be assessed so that all potential problems can be solved immediately.

Table 1. Inappropriate forestry management methods in catchments populated by FPM and their negative impact on the catchments.

Inappropriate management method:	Negative impact:
Monodominant spruce stands of the same age.	Acidic needles lower water pH, trees near watercourses shade and cool down the water, unsuitable conditions for production of food for FPM.
Large-scale use of insecticides, pesticides, and herbicides in pearl mussel catchments. Application of game repellents.	Risk of acute toxic pollution of the environment, decline of a specially protected species, must always be discussed with the nature conservation authority.
Burning wood brash close to a watercourse (closer than 10 m).	Rapid deterioration of water chemistry due to ash being washed into watercourses.
Fuel leaks	Source of toxic chemicals.
Branches and bark blocking watercourses and drainage channels during harvesting.	Risk of formation of toxic chemicals (ammonium ions, phenols, etc.).
Storing wood, piles of bark, wood chips, and other harvest residues on waterlogged land.	Risk of leaching phenols.
Storage and drainage of water from the road network near watercourses.	Washing alluvia into watercourses, turbidity.
Reinforcing transport roads with bundles of branches (fascine roads) on waterlogged land.	Leaching of timber, development of chemicals, which are toxic for FPM.
Leaving felled trees in watercourses.	Branches and bark blocking watercourses, interruption of catchment continuity and consequent reduction of food supply for FPM, loose tree trunks may damage FPM colonies.
Mechanical or biological damage to soil during harvesting near a watercourse, in particular on steep terrain.	Increased erosion, excessive washing of stream load into watercourses, directly threatening live pearl mussels, in particular in the young stages of development.
Movement of heavy machinery in waterlogged stands.	Increased erosion.
Crossing watercourses with heavy machinery.	Turbidity, increased erosion.
Use of concrete, galvanized, coppered, or otherwise treated culverts for routes crossing watercourses.	Toxicity of heavy metals leached to the catchment from construction.
Transportation roads and dry ditches drained directly into watercourses.	Turbidity and washing sediments into the catchment during rain.
Clearcutting on steep terrain.	Risk of erosion and pollution with sediments.

Wood should only be dumped in permanent landfill sites with reinforced surfaces (using a granulite gravel bed with coarse fragments at the bottom and finer fragments on top). Outside such sites, wood brash can only be stored provided that the wood will not leach, meaning that it must not lie in water, not even temporarily (because of the danger of phenols leaching into water). Piles of bark, wood chips, or other harvest residues located on waterlogged land pose a similar risk.

Also it is advisable to avoid water retention and draining on timber harvesting routes and forestry transportation roads, especially if the road network is situated close to watercourses. For this reason, it is essential to reinforce the areas where timber harvesting routes meet forestry transportation roads, using granulite gravel when creating new timber harvesting routes or reconstructing old ones. This should be done:

- at least 10 metres into land with a gradient of 5 % (elevation of terrain in 100 m has been considered)
- at least 20 metres into land with gradient of more than 5 %.

Reinforcing forestry transportation road surfacess with granulite gravel is recommended in order to prevent water retention. Reinforcing transportation roads with fascines (bundles of branches) poses a risk of wood leaching and consequent release of substances toxic to FPM. This occurs as a result of the reinforced section soaking in water accompanied by decomposition of the wood brash. Therefore, fascine roads can only be used provided that the forestry transportation road is drained by means of percolation to the forest cover.

Use of concrete pipes, concrete grade slabs, and other concrete elements in water should be avoided. Water in FPM catchments usually has a very low content of solutes (conductivity is often less than 40 µS/cm). These aggressive waters rapidly wash the binder out of concrete and shorten the lifespan of the elements as well as the structures. An increased concentration of calcium in water is problematic for the FPM as a calciphobic species.

During harvesting and transportation of wood brash from a stand, it is crucial to prevent any fuel and lubricant leaks from the machinery.

Water Regime Protection

As a part of forestry management, it is crucial to support small wetlands in the stands as an **anti-drought measure** (water regime protection measure) (**Fig. 95**); this, however, does not apply to small ponds and catchments that capture flowing detritus.

The result should be a sparse and irregular structure of forest cover in valleys and places with a natural outflow of water – helocrenes. It is recommended to open up stands along watercourses and spring areas (**Fig. 96**, **97**). Felled trees must be removed and the wood brash must not be left in the watercourse. It is necessary to proceed carefully and use appropriate equipment so that neither the soil nor the watercourse are disturbed.

Protecting the water regime against the stream load is a priority issue in logging work for both incidental and planned logging. Stream load occurs as a result of increased erosion in places where **the soil profile has been damaged, mechanically or biologically**. Irreversible loss of the top soil layers prevents subsequent restoration of the original soil properties at the given location and, at the same time, **the fine alluvia destroy the FPM's environment**.

Particular attention should be paid to activities carried out on sloping terrain. Harvesting procedures and timing should be assessed and, more importantly, checked. Harvesting and planting works requiring movement of the wood brash should be carried out when the watercourse is frozen. **The use of heavy machinery in waterlogged areas should be excluded.** The wood brash may

Fig. 95. A small wetland shaded by forest cover.

be removed using horses or with ropes from outside the waterlogged area. Heavy forestry equipment must not be used outside forestry roads.

The same rule also applies for crossing watercourses with heavy equipment. Crossing watercourses and their tributaries in unpaved places causes water turbidity, either directly due to the crossing itself, or indirectly, when the watercourses or tributaries are disturbed and consequently redirected to the transportation route (**Fig. 98**). The preferred option is to transport timber parallel to a watercourse, as high above the thalweg as possible; in the case of temporary crossing of a watercourse, the banks should be stabilized with horizontally laid tree trunks, which are removed after harvesting. For intersections of a watercourse and a skid road or a similar road it is recommended to build a crossing from debarked beams (only where possible

Fig. 96. Demonstration of appropriate management – an open stand with a gully in the middle.

Fig. 97. Selection cutting in a stand resulting in opening up a watercourse.

Fig. 98. The consequences of using heavy machinery in a waterlogged stand.

with regard to the profile of the terrain – the beams must not lie in water) or to redirect the watercourse into pipes leading under the road (for minor capillaries the road can be bridged by an open wooden channel). Bridges made of round logs can be also used. All crossings must be removed after harvesting (this includes decaying bridges from previous harvesting works, which may collapse and block a watercourse). The use of plastic materials is advisable for construction of culverts (alternatively, steel pipes may also be used, but always without applying of tar or other protection). Concrete materials, galvanized or copper-plated materials, or materials treated with a combination of galvanizing and copper plating must not be used because of the toxicity of the metals for bivalves and fry of all species of fish. Construction and reconstruction of timber transportation roads, including construction of bridges and culverts on a forest watercourses, can only be carried out with the approval of the relevant conservation authority.

During planned harvesting, as well as random harvesting carried out on a larger scale, active promotion of sedimentation of the water affected by erosion is important (**Fig. 99**). Turbidity can be reduced by temporary overflow of the watercourse to the stand, where possible due to less steep terrain. It is recommended to build small sedimentation tanks under forestry road ditches, which are directed from the slope towards the watercourse, and to clean these tanks after excessive rain. The use of soak pits is suggested where mud or leachate may be transported by a watercourse to a FPM habitat and where, at the same time, a transportation road is running through an area covered by herbaceous vegetation. **Transportation roads and additional dry ditches cannot be drained directly into the watercourses**.

Eroded potholes and gullies in a forest (**Fig. 100**) often occur in stands with unfavourable species composition following inappropriate harvesting (such as clearcutting on a steep terrain) or after windthrow, and in locations with increased erosion. Gullies are a long-term source of stream load. However, stream load is not produced solely by water erosion during periods of increased flow rate. It also develops as a result of frost erosion of the steep slopes of gullies or banks which are not properly stabilized by roots. During the spring months, a larger network of forest drainage ditches located in the spring area can fill the entire cross section of the watercourse with sediment, with a consequent rise in water level and undermining of the banks, even in steady flow. Undermined banks gradually collapse and this new supply of stream load is transported to the main stream. The combined erosion damage thus affects long sections of a stream bed. A suitable solution for such situations is to divide a gully or pothole by a system of willow-alder grids combined with riprap, as needed. More information is available in **Chapter 10**.

Fig. 99. Sedimentation pool created after harvesting.

Fig. 100. Erosion gully in a stand.

8.1.2 Non-forest Land

Stands in these areas do not have the character of a forest and, in most cases, grow on non-forest land; therefore, **this land does not fulfil the function of a forest**. They include:

- *permanent non-forest areas* (stands of different ages covering a maximum of 20 % of the area);
- *waterlogged meadows and spring areas*;
- *waterlogged areas on floodplains*;
- *large helocrene springs*;
- *riparian stands*;
- *forest stands* on land which is not intended to function as a forest.

Following the cessation of agricultural activities (mowing, grazing) during the 20th century in many localities with FPM, formerly open areas were gradually colonised by woody plants, often spruce. This poses a risk of shading of small watercourses, which had been previously flowed through meadows. In addition, there is the threat of development of an acid layer on the surface of the soil due to needle fall, as well as a decline in the herbaceous vegetation in spring areas and floodplains. In such areas, intervention in terms of species composition of woody plants may be considered. Keeping the areas open is also beneficial with regard to the population of *Lyrurus tetrix* (black grouse) and *Tetrastes bonasia* (hazel grouse), as well as enhanced food supply for deer.

Riparian stands along watercourses

For riparian stands of large or small watercourses, or spring rills managed by the state river basin enterprises, Forests of the Czech Republic, or other land owners), a structure of sparse and irregular stands with gaps and natural species composition should be preserved or established in catchments populated by FPM (**Fig. 101**). It is necessary to eliminate shading by tall spruce stands by means of planting or harvesting. It is recommended to plant small groups of woody plants, which are missing from the natural species composition in existing or artificially created gaps. It is advisable to cut young trees and shrubs with a brushcutter while this is still possible. Any woody plants that fall into the watercourse should be removed immediately if this is required by the Water Act (in particular this applies to larger watercourses and if (a) transverse constructions on the watercourse are under threat, (b) dams occur on the watercourse, and (c) there is a possibility of endangering meanders). With regard to protecting FPM, it is necessary to remove especially those fallen trunks that directly endanger mussel colonies or that can block the watercourse or damage the banks and consequently cause excessive sedimentation of water.

Spring areas and large helocrene springs

In spring areas (particularly helocrene spring areas), quality detritus develops (decomposed organic material washed by streams into areas populated by FPM serves as a source of food). In order to support natural development of quality detritus, it is advisable to open up stands of coniferous trees, which are shading helocrene spring areas, resulting in changes to the composition and vegetation cover of the spring area (**Fig. 102, 103**). In contrast, partial shade is characteristic for the spring areas covered by *Cardamine amara* (large bitter-cress). A general principle of preservation of quality spring areas in river catchments is not to interfere with them – do not to drain them or modify their relief because such interventions may lead to their complete destruction.

Fig. 101. Scattered willows along the Teplá Vltava.

Fig. 102. Spring area, partially shaded by naturally regenerated trees.

Permanent non-forest areas

Permanent non-forest areas must not be planted or drained. In fact, it is necessary to regularly reduce the number of trees occurring as a result of natural regeneration so that the non-forested area would not become covered by woody plants (**Fig. 104**). Therefore, browsing of the plants by game is desirable in waterlogged localities.

Forest stands on non-forest land

If hardwood trees are present on non-forest land as a consequence of natural regeneration, the composition of the species can be easily and significantly adjusted by cutting down some of the spruce trees. Desirable tree species can be planted in suitable locations – these include *Alnus glutinosa* (common alder) up to 700 m above sea level, *Alnus incana* (grey alder) at higher altitudes, *Ulmus glabra* (wych elm) on sloping spring areas outside frost hollows, *Acer pseudoplatanus*, *Fraxinus excelsior* (European ash), and *Betula pendula* (silver birch). Birch trees are more suitable as solitary trees because in recent years they have not been able to withstand heavy snow in warm winters and consequently they are rapidly disappearing, even from the areas where they previously flourished. *Prunus padus* (bird cherry) is not suitable because its dense growth makes it difficult to maintain the flow and contributes to occurrence of wood debris in the watercourse. Planted individuals must be protected against browsing without the use of chemicals.

In particular for young stands along a watercourse, thinning of stands is recommended to carry out thinning of the stands up to 10 m from each bank, thus creating and preserving permanent non-forest areas. However, the structure of the riparian stand should remain sparse and irregular. Small-scale measures are suggested to create such a structure in densely covered areas. It is recommended to create small openings as space for natural regeneration. In contrast, large-scale measures to achieve a sparse structure are not advised because they pose a risk to the mechanical stability of

Fig. 103. Spring area after removal of naturally regenerated trees.

stands. An ideal option is sparse and irregular structure (**Fig. 105**). This can be achieved by asymmetric interventions of varying intensity, such as working on smaller areas with a gradual decrease of intensity away from the watercourse (very intensive interventions in young stands should be carried out along the watercourse). Appropriate forest management methods for forests o non-forest land should be implemented in accordance with EU and local legislation.

8.2 Management principles and recommendations for hunting

Hunting in itself does not have a negative impact on FPM populations. However, indirect damage may occur by damaging hardwood trees (after natural regeneration or planting); this happens in particular in areas where game animals stay permanently. Individual tree protection or game-proof fences are therefore necessary in order to introduce suitable of hardwood tree species. Large-scale application of repellents in the proximity of watercourses is not suitable.

It is also important to assess the suitability of locations for feeding racks, baiting site, mineral feeds, medicated feeds, etc. Sites, which are close to watercourses and spring areas are not suitable (the minimal recommended distance of such locations from a watercourse or spring area is 30 metres). In particular, significant wetland communities are endangered by higher levels of eutrophication and mechanical damage to the soil and vegetation caused by an increased concentration of game in the area, or by introducing ruderal and non-original plant species from seeds contained in their feed.

Fig. 104. Gradual covering of a non-forest area with naturally regenerated spruce in the upper part of the Blanice River catchment in Boletice military area.

8.3 Management principles and recommendations for agriculture

Agriculture has always been related to the occurrence of FPM, especially at higher altitudes (today's upper limit of its occurrence). Agriculture in the form of livestock farming and meadow management created new ecological niches for FPM in the areas with otherwise unfavourable conditions, which allowed this species to survive the period of increased anthropogenic stress to the present day. Therefore, it would be inappropriate to completely eliminate agriculture in catchments populated by FPM. This would lead to decline of the mosaic character of the landscape and open floodplains, and to cooling down of the river network following large-scale expansion of forests. Local farmers and owners of agricultural land are thus significant partners for nature conservationists.

8.3.1 Appropriate cultivation methods

The management methods applied in a catchment are of crucial importance for the protection of FPM. The following cultivation methods in particular are considered inappropriate: (a) methods increasing soil erosion and its run-off into water bodies (use of heavy machinery in waterlogged areas, arable land on slopes with an inappropriate gradient); (b) methods which decrease biodiversity of the landscape (large plots of land, intensive permanent grassland) or its retention capacity (construction of drainage systems). Point sources of pollution are also problematic (cesspools, dung heaps in fields, silage pits or silage bags near watercourse). Pollutants washed out from these sources to a watercourse may cause short-term acute toxicity of the environment, which is lethal for FPM. Particular attention must be paid to the use of fertilizers and biocides, or to only use fertilizers and biocides,

Fig. 105. Semi-open area with a natural composition of woody plants in the Spálenecký potok stream catchment.

which are certified as harmless for the subject of protection (FPM). Inappropriate management methods in the FPM catchments and their negative impacts are listed in **Table 2**.

Organic farming, especially in the form of meadows livestock, is the optimal management option in catchments with FPM. In such catchments, the cultivated land should constitute a varied mosaic of extensively harvested grassland in dry areas and unused wetlands or spring areas. Moreover, extensive forms of agriculture, which use organic fertilizers are a prerequisite for development of quality detritus, which is a source of food for FPM.

Table 2. Inappropriate agricultural management methods in catchments populated by FPM and their negative impact on the catchments.

Inappropriate management method:	Negative impact:
Use of tractors and other machinery in watercourses or spring areas, crossing watercourses with machinery.	Increased erosion, transport of sediments and alluvia, disturbing hydrological regime, risk of fuel or gas leaks into the water environment.
Storing mown biomass (mulching) on waterlogged meadows.	Source of nutrients and harmful substances increasing the volume of nutrients in the catchment.
Free movement of cattle and sheep in spring areas and near watercourses.	Destruction of springs and spring gullies, increased erosion, occurrence of incised stream bed in the upper sections of FPM streams.
Growing broad-leaved crops close to watercourses or on steep terrain.	Increased erosion, leaching of nutrients and pesticides into watercourses.
Turning temporarily grassland into arable land and rapid restoration of meadows close to watercourses or on steep terrain.	Increased erosion, rapid release and leaching of accumulated natural nutrients into watercourses.
Use of biocides, desiccants, fertilizers, and substances for stimulation of crops less than 30 m from spring areas, banks of watercourses, and edges of other wetland areas.	Increased toxicity and supply of nutrients into FPM catchments.
Accumulation of large masses of mown grass from individual properties in ditches and hollows near watercourses.	Source of nutrients and harmful substances increasing the volume of nutrients in the catchment.
Storing fertilizers, silage, and compost near watercourses.	Source of nutrients and harmful substances increasing the volume of nutrients in the catchment.
New draining of agricultural and forest land.	Decreased retention capacity of the landscape, rapid increase in conductivity, great risk of erosion or nutrient wash-off from area with a high volume of nutrients.
Using chemicals for cultivation of land, which have not been proved to be harmless to juvenile development stages of FPM.	Toxicity of the environment, direct loss of a protected species.

8.3.2 Livestock farming

In catchments populated by FPM it is recommended to introduce and support an extensive livestock farming. Intensive grazing (especially on steep land) in the proximity of watercourses and spring areas causes extreme pollution by nutrients. An environment with low content of nutrients, which is suitable for FPM, is thus endangered by eutrophication. The loading of the soil with nutrients should be closely monitored; in smaller pasture areas, and if the load is excessive, appropriate measures should be taken. For example, timely relocation of a mobile water trough can prevent excessive damage to the turf and point accumulation of nutrients close to watercourses, which could subsequently be washed off into the water. Watercourses and wetlands should always be separated from pastures by a fence (**Fig. 106, 107**). It is necessary to consider the fact that the amount of phosphorous and nitrogen produced by cattle is high due to their intensive metabolism, whether they live in sheds or on pastures. The balance of phosphorus and nitrogen production of one dairy cow corresponds to the production of 32 equivalent inhabitants (Kaminský 1974). An equivalent inhabitant is a unit corresponding to the production of wastewater by one person. Feeding areas and wintering grounds should also be located away from wet and waterlogged sites, and at a sufficient distance from the thalweg. Even a single concentrated infiltration of nutrients and sediments into a watercourse can endanger the young developmental stages of FPM.

The load of land grazed by cattle and sheep has a significant impact on the balance of nutrients on a pasture in the vicinity of a watercourse with FPM, and consequently on the food supply for the mussels. As a limit of agricultural use of grazed areas, a value of 0.5 livestock units (LU) per actual grazed hectare at the current herd size up to 100 animals should apply, with the optimal value of about 0.35 LU/ha (depending on the location) and the size of the herd up to 50 cattle and 100 sheep. However, it is always advised to discuss the needs of the farmer and nature conservation with the relevant local nature conservation authority. More information on grazing options in specially protected areas and Sites of Community Importance in Czechia is available online in the **Standard of the NCA CR** – Livestock Farming: http://standardy.nature.cz/schvalene-zneni-standardu/.

Fig. 106. Well-organized grazing of cattle on dry slopes near Zbytiny in Šumava Mountains, with fencing around the watercourse.

With regard to livestock farming, cattle owners in particular should ensure proper fencing of pastures and subsequent regular inspections. Regularly cutting the grass under fences and electric fences is advised; however, Roundup must not be used because of its toxicity to FPM. If cattle escape from a pasture and wander into a spring area, the spring areas and gullies supplying FPM with food can be destroyed. Damaged banks of watercourses are prone to gully erosion and, as a result, the watercourse is filled with sediments that settle further downstream and threaten the young development stages as well as the adult FPM. Pasture owners can contribute to the protection of FPM by fencing wetlands and spring areas connected to a watercourse within their pastures. Also, it is recommended to provide crossings for the cattle moving from one pasture to another, for example with a wooden bridge, so that the water is not filled with churned sediments. For heavily eroded watercourses running through pastures, redirecting the flow to the soakage system should be considered.

8.3.3 Meadow management

Support and maintenance of extensive meadows with a network of small streams and gullies is desirable in order to provide detritus to the FPM in the catchment. One of the ways of developing detritus, which is the main source of food for mussels, is leaching soil particles from the root systems of certain types of meadow vegetation. The different types of suggested management measures suitable for such habitats are listed in **Chapter 9**. If possible, the owner of the meadow should assess the character of the meadow habitat (waterlogged, dry, with low volume of nutrients) with regard to the use of meadow vegetation. For instance, in some meadows with a lower volume of nutrients which are within agroenvironment grant programmes and which are mowed twice a year, weeds are spreading (in particular *Hypericum perforatum* – St John's wort), which has often led to use of herbicides (Roundup). Contamination of water with this type of herbicide may cause a decline in the FPM population. At the end of 1990s, deaths of juvenile FPM from rescue rearing (and probably also from the wild population) were documented after application of Roundup.

Fig. 107. Negative impact of intensive grazing (soil poaching and compaction) near a watercourse after herding cattle across the pasture in Zbytiny.

8.3.4 Fertilizer application

Organically bound nitrogen contained in organic and organomineral fertilizers, livestock manure, and treated sludge is mineralized in the soil and transformed into forms usable by plants. However, at the same time, the long-term presence of nitrogen in the soil causes a disbalance of nutrients and a loss of organic mass, and nitrogen is leached into the environment (Záhora et al. 2011). The classification of fertilizers according to their nitrogen release rate is listed in **Table 3**.

When applying fertilizers to arable land it is necessary to consider:

- a) ***time of application*** – slurry application during winter (**Fig. 108**) is generally prohibited in our conditions,
- b) ***land gradient*** – application of nitrogen fertilizers on arable land with gradient of more than 10° (17.36 %) is not advised (more information on fertilizer use on steep terrain close to surface water is available in **Annex 10**),
- c) ***type of land*** – use of fertilizers on waterlogged agricultural land should be excluded,
- d) ***proximity of watercourse*** – in FPM catchments, the minimum distance of fertilized land from the edge of spring areas, banks of a watercourse, and other wetland areas should not be less than 30 m,
- e) ***fertilizer type***.

The storage of fertilizers should also be considered. In catchments populated by FPM, fertilizers should be stored as far from the watercourse as possible, and should always be secured against leaching into the catchment.

Fig. 108. Inappropriate use of slurry on arable land in winter, last documented in the Blanice River catchment in 2003.

Table 3. Classification of fertilizers according to the rate of release and possible leaching of nitrogen (Klír & Kozlovská 2012).

Fertilizers with rapid release of nitrogen	Fertilizers with slow release of nitrogen
manure slurry fugate of slurry liquid manure manure silage effluent faeces from poultry and small farm animals faeces and urine left on pasture by livestock digestate from biogas stations fugate of digestate	cattle or pig manure horse manure slurry separate compost separated digestate

Mown agricultural land can be fertilized with composted manure, provided that manure heaps are located outside the catchment. For small agricultural production or gardens, it is recommended to create turf piles on which mown grass can be composted. After the end of the composting cycle, the composted turf can be used for horticulture.

More information and recommendations regarding fertilizer application is available in the methodology of the Ministry of Agriculture of the Czech Republic (Kozlovská et al. 2020).

8.3.5 Recommendations for conservation managers on agricultural management issues

Conservation managers are advised to use government grants to encourage meadow management and cattle breeding in catchments with FPM, but only if the aforementioned activities are done in accordance with the principles of organic agriculture specified in the standards issued by International Federation of Organic Agriculture Movements and inspected by national certification organizations. An optimal option is organic farming by local farmers or co-op members who, unlike the large joint-stock companies (which cultivate rented land), have an interest in the sustainable use of their land and a personal relationship to the land. An example of systematic classification of land management zones in the proximity of watercourses which are located in Specially Protected Areas (SPAs) can be found in **Annex 11**. This classification may also serve as an example of how to use the land in FPM catchments outside an SPA, as agreed between the owners of such land and nature conservation authorities.

8.4 Management principles and recommendations for fishermen

Fishery management in river catchments populated by FPM should primarily focus on maintaining a stable population of *Salmo trutta* m. *fario* with a sufficient number of fish of each generation (Švanyga et al. 2013). An optimal solution is to declare FPM sites as fish protection areas. Also, local branches of the Czech Fishing Association should cooperate with local nature conservation authorities. It is important to establish a regime friendly to FPM in fish protection areas. For example, if the fishing grounds serve as a place for catching fish of a certain generation, it is necessary to determine when and where fishing should be allowed with regard to the occurrence of FPM population.

A suitable approach to catching *Salmo trutta* m. *fario* in adjacent fishing grounds is to increase the required commercial size or, if necessary, to extend the close season. For example, it is recommended to introduce the "catch-and-release" form of recreational fishing.

In locations populated by FPM, wading in rivers must be strictly prohibited, either for the whole fishing grounds or within clearly marked sections (at least 300 m long) so that FPM colonies are not damaged (in catchments with FPM it is advised to direct the fishing to the areas demarcated under a first watershed outlet).

8.4.1 Increasing local *Salmo trutta* m. *fario* populations

In places with low populations of *Salmo trutta* m. *fario*, increasing these populations by means of artificial but genetically localized breeding is suggested (Švanyga et al. 2013). It is important to realize that the parasitic larvae stages of FPM develop primarily on young individuals (1 to 2-year-old fish). Older fish develop immunity against further parasitic infestation after the first glochidia attack and are difficult to exploit for FPM from the reproduction point of view.

This, however, only applies to unnaturally influenced habitats with extensive fish stocking without a sufficient impact of predators. In Blanice National Nature Reserve, where there is good natural reproduction of the trout population and permanent predation pressure from *Lutra lutra* (Eurasian otter) and *Ciconia nigra* (black stork). Larger trout of about 14 to 18 cm body length seem to be the best hosts for glochidia due to their large gills, where the glochidia are well-enclosed and thus develop as more healthy individuals. These larger fish enter the main watercourse from cooler tributaries where FPM do not live and, as a result, they are not immune against glochidia (Hruška 2000a). Therefore, natural representation of all fish age groups in the *Salmo trutta* m. *fario* population is a desirable effect of the influence of predators (*Lutra lutra*, *Ciconia nigra*). However, these predators also need other sources of food, which remove some of the undesired predation pressure from the fish population. Therefore, it is important to have sufficient suitable habitats populated by amphibians, reptiles, small rodents, and insects near watercourses.

Artificial increasing *Salmo trutta* m. *fario* populations in a catchment can be carried out, for example, with Firtzlaff's hatching apparatuses located in a watercourse (**Fig. 109**), or stocking with young fish raised outside the watercourse; but the fish must always be locally bred (Spisar 2015). However, the long-term goal of these supportive activities should be to create independent and naturally reproducing *Salmo trutta* m. *fario* population.

8.4.2 Maintaining a suitable habitat for *Salmo trutta* m. *fario*

A good measure is to open the canopy and purify a stream so that both the main watercourse and small tributaries are opened up, thereby increasing the trout breeding capacity of the watercourse and, at the same time, removing obstacles limiting detritus transport to the main watercourse and to FPM populations. Removal of undesired brash wood must be done carefully, without using heavy machinery in the watercourse, and with a minimal impact on adjacent floodplains. *Salmo trutta* m. *fario* occurrence in a watercourse can also be supported by building artificial fish shelters (**Fig. 110**) or by inserting dead wood, resulting in the development of pools – breeding capacity depends on the chances of finding shelter.

In catchments populated by FPM, making pools in order to create favourable conditions for fish fry is always problematic. The most appropriate way of doing so is building spur dikes, which slow

Fig. 109. Floating Firtzlaff's hatching apparatuses in a watercourse (Czech Fishing Association in Aš). Based on acquired experience, it can be confirmed that fish bred this way have a lower mortality rate and, it is assumed, trout will return to the location in order to spawn.

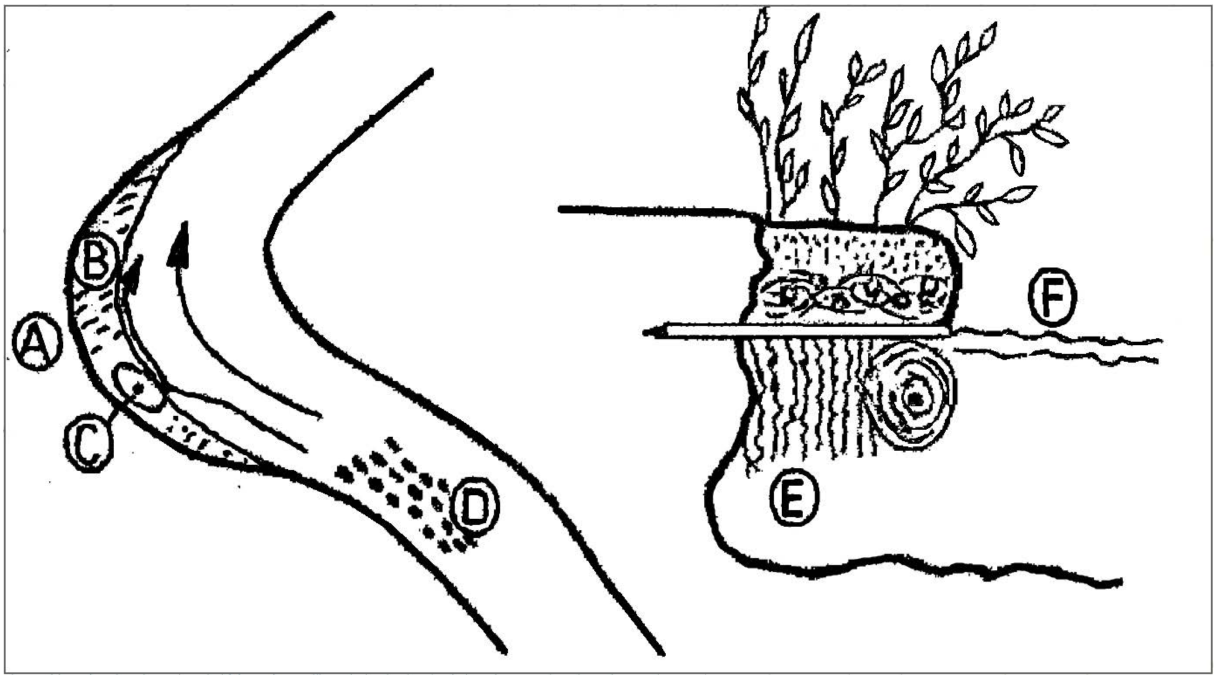

Fig. 110. Modification of banks in order to increase the number of opportunities for glochidia to attach onto host fish. A – eroded bank, B – vegetational adjustments resulting in an overhanging bank, C – swirling currents with glochidia, D – FPM colonies, E – fish shelters, F – medium water level. After Hruška (1995).

down the flow but do not fully dam the watercourse. With regard to the migratory possibilities for trout, this kind of measure should primarily be applied downstream from FPM sites; transverse obstacles on the flow above their habitat cause retention of detritus, which is an important food source for FPM.

Introduction of competitively stronger species of fish ('white fish') and non-native species (*Salvelinus fontinalis* – brook trout, *Oncorhynchus mykiss*) is particularly inappropriate; they often push *Salmo trutta* m. *fario* into tributaries, thus leading to its absence during FPM breeding. Excessive predation pressure on trout populations caused by migration of predatory fish is also undesirable (for example migration of *Aspius aspius* from Lipno reservoir into the Teplá Vltava River). Fish stock plans for FPM localities should always be discussed with nature conservation authorities.

8.4.3 Construction and creation of water works on a watercourse

Building of river ponds or reservoirs (built on the main stream) with a dense fish population is not advised in catchments populated by FPM. In general, ponds increase the volume of nutrients in oligotrophic watercourses, as well as the pH value, thus increasing the toxicity of leached ammonium ions. Moreover, river reservoirs completely retain detritus carried by water, which is a source of food for mussels. Ponds may have a negative impact on the composition of the fish population due to fish moving downstream, and may also clog the bottom of the watercourse with excess organic mass, mainly developed from phytoplankton. The negative impact is generally smaller for side ponds (built beside the main stream, connected to it by input and output channel). If undesired fish species regularly escape from breeding ponds into watercourses with FPM, appropriate mechanical barriers (such as screens) may be installed in the inflow or outlet areas.

Construction of fish passes at existing weirs is recommended. However, preparation of each project must be discussed in advance with the relevant conservation authority. Construction of small hydro power stations on watercourses should be prohibited in FPM areas (above the first watershed outlets).

8.4.4 Recommendations for conservation managers regarding fishery management issues

For conservation managers, communication with fishing associations and fishermen is crucial with regard to fishery issues in watercourses populated by FPM. It is recommended to inform the public about natural predators of *Salmo trutta* m. *fario* (e.g., *Lutra lutra* and the relationship of FPM and *Salmo trutta* m. *fario* in general). This goal can be achieved by using educational materials, organizing educational seminars, public events, or interdisciplinary meetings, and via regular contact between nature conservation representatives (representatives of public administration, specialized non-profit organizations, etc.) and representatives of the local fishermen and hunters (e.g., via annual meetings).

8.5 Management principles and recommendations for municipalities

For municipalities whose cadastral areas include watercourses populated by FPM, the principles of management and recommendations stated in the management plans of individual protected areas and their protection zones are well applicable. For FPM, management of the aquatic environment is a priority (i.e., rivers, streams, rills, and spring areas), especially in comparison to “dry” parts of landscape which only influence water indirectly. The important task for municipalities is to support water retention in the landscape; however, correct wastewater treatment and solid toxic waste treatment are equally important.

8.5.1 Wastewater treatment

In larger municipalities, wastewater must be drained by the sewage system into wastewater treatment plants (WWTP) with a sufficient degree of treatment. For municipalities, it is necessary to carefully monitor the process so that any problems or large leakage accidents are prevented in time. In the case of accidents, it is advisable to prepare alternative methods of wastewater disposal so that the wastewater does not leak into streams populated by FPM. The best solution is construction of two low-loaded water reservoirs in line following the discharge from the WWTP as the third degree of post-treatment (**Fig. 111**) and to drain the water into the watercourse from the second reservoir. The emergency/rainwater outflow of the WWTP must also be drained to the first reservoir, not to the

Fig. 111. Aerial view of low-loaded biological ponds for post-treatment in Zbytiny Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

stream. The third degree of treatment increases investment costs but ensures long-term and almost 100 % operational safety (in an emergency situation, water from the WWTP or sewage system will be captured by the first reservoir) with a good level of efficiency. The operational costs will not rise significantly and the reservoirs can also have secondary uses. With the average number of failures of sewage technology, mud removal from the first reservoir can be expected in about 10–20 years (and in 30–100 years for the second reservoir). Also, the second oxidation reservoir can be used for breeding of carp family species (those that will not survive in the watercourse if they escape from the reservoir) and also (considering the normal operating depth of 0.5 m) as a safe ice-skating rink in winter (Wanner & Simon 2012, Wanner et al. 2012, 2014, 2015). Fishing can help to remove nitrogen and, above all, phosphorus from the system; however, fish in the reservoir must not be fed. The efficiency of inclusion of post-treatment ponds in wastewater treatment systems is shown in **Figure 249** in **Annex 12**.

For individual construction works, the specific character of the locality must be taken into consideration with regard to wastewater disposal, and any possible pollution of the surface water must be prevented, even if it seems insignificant. In the summer dry season, even a single house or cottage can pose a significant threat to small watercourses with low flow rates. If there is enough space, the system of low-loaded reservoirs (domestic WWTP and two small ponds in line) is suitable for individual construction (**Fig. 112**, **113**; Wanner et al. 2014).

Fig. 112. A small post-treatment pond for five equivalent inhabitants next to a farmhouse in Malovice near Vodňany, adjacent to a domestic WWTP shortly after being built (a simple in-ground reservoir in argillaceous soil). The first of two low-loaded biological reservoirs.

8.5.2 Solid waste disposal

Activities, which could have a negative impact on the protected species due to water pollution, include storage of industrial and municipal waste near a watercourse, but also on any waterlogged land within a river catchment. From this point of view, illegal dumping pose a risk, as do neglected areas on private and municipal land. Therefore, it is essential to ensure proper disposal of household waste and the periodic collection and transport of hazardous waste within municipalities.

The accumulation of larger quantities of seemingly harmless mown grass in ditches and hollows around individual properties is also a problem. Large amounts of rotting phytomass produce a lot of ammonium ions and, after rain, the leachate that develops can be a source of harmful substances for the aquatic environment, fish, and FPM. Municipalities should provide secured areas for composting waste grass, which can later be used as a fertilizer. A good solution to the problem for individual properties is to create a soil pile at least 0.5 m high for composting mown grass. Three years later the composting soil pile can be mixed together with the decomposed grass and used as a quality garden fertilizer. Likewise, a smaller volume of grass mass from gardens can be put on conventional compost, always mixed with drier carbon-rich material such as straw, leaves, twigs, or wood chips.

8.5.3 Recreational use of land in catchments with freshwater pearl mussel

Long-term stays and group forms of recreation should be directed away from the thalwegs of watercourses populated by FPM, especially where there is a risk of erosion. Sport and recreational activities, which do not disrupt the balance of nutrients and do not aggravate erosion processes in sensitive areas should be given preference. This also applies to erosion caused by activities such as cycling (and cross-country skiing in winter).

Fig. 113. The same reservoir (see Fig. 112) one year after construction: wetland vegetation and turbidity caused by unicellular algae are gradually developing. This contributes to better post-treatment.

Scout camps or summer camps for children are possible provided that there is sufficient distance of the toilets and kitchen soak pits from a watercourse. Any permanent camp sites with buildings are problematic because of the issues related to wastewater treatment. Restrictions in protected areas also apply in order to protect other sensitive animal species (*Tetrastes bonasia*, *Ciconia nigra*, *Lutra lutra*, *Crex crex* – corn crake, etc.).

8.5.4 Transport and other infrastructure

Reconstruction and maintenance works on the road network should be carried out in a manner that does not have adverse effects on the aquatic environment.

For transverse structures (bridges, weirs, fords), reconstruction or construction works near watercourses, which could have negative impact on the subject of protection, it is necessary to provide close surveillance of the physical and chemical parameters above and, in particular, below the construction site, with continuous measuring of conductivity and reporting if the indication value is exceeded. These works can also be carried out by the person in charge of biological monitoring.

From the point of view of the road network, not only fuel leakages, but also salting of roads (using solid salt or spraying the road with salt solutions) and the use of crushed aggregates with a high calcium content as a spreading material or for reinforcing roads can have a significant negative impact on water. Herbicides for the treatment of roads or railway tracks also have a similar negative impact if they are harmful to water.

Drainage ditches alongside roads should drain into soakage systems and not into watercourses and spring areas. For agricultural roads near watercourses, it is recommended to consider reconstruction of existing culverts with soakage systems or construction of new culverts, which will not drain directly into a watercourse. One of the functions of culverts is separation of the hydrographic network from water contaminated due to cattle crossings. A culvert can be built using a plastic or steel pipe (use of concrete pipes should be avoided), which is then reinforced by rock riprap. Under the pipe, the ditch should be closed by tamped soil and the surface of the riprap should be covered by turf. Such a short section of piping does not deteriorate detritus and does not affect the water temperature.

Particular attention must be paid to construction of roads, transport routes, skid trails, etc. Roads must always cross watercourses on the strongest terrain and on solid bridges. Plastic or steel pipes can be used for construction of small culverts, whereas for larger bridge constructions it is also possible to use concrete prefabricated elements that allow preservation of the natural river bottom. During reconstruction of transverse structures on a watercourse, measures must be taken to prevent negative impact on water quality must be taken. For example, the fronts of culverts can be primarily reinforced with dry stone walls or heavy rock riprap; alternatively some other reinforcing elements may be used, such as geogrids (but they must not be galvanized).

8.5.5 Principles and recommendations for construction work

It is important to note that water pollution may occur during any construction activity (new construction, reconstruction of bridges and other transverse structures over watercourses). Concreting in particular should be avoided. Biological monitoring must be provided for all such construction activities. The person in charge of monitoring should test water quality (e.g., using continuous measuring of conductivity above and below the construction site with automatic reporting of potential exceeding of values). Only local aggregate, stripped of clay fractions, should be used and measures should be taken in order to eliminate calcium or toxic leachate from construction material.

Crossing watercourses with pipelines (e.g., dive culverts, sewers, gas pipelines) or cable lines is also a significant threat to a watercourse. During construction or in the event of an accident, the stream may be directly contaminated. In order to lower such a risk, sealing the excavation with bentonite dividers can be used. This technology have been successfully used, for example, for protection of the FPM population in Zlatý potok stream catchment. The excavation thus cannot drain liquids leaking from the piping into the watercourse. At the same time, this measure permanently eliminates undesired drainage of shallow groundwater from buildings or arable land, which is often heavily loaded with nutrients.

Please note that any plan whose implementation requires intervention in a watercourse and its surroundings, spring areas, and so on must be approved by the relevant conservation authority. It is always better to prepare the project in cooperation with the nature conservation authority and to implement it only after the necessary exemptions have been granted according to the relevant legislation.

Chapter 9: Meadow management in areas with freshwater pearl mussel

Author: RNDr. Alena Vydrová, Jaroslav Hruška, Mgr. Anna Kladivová
Reviewers: Mgr. Michala Mariňáková, RNDr. Ivana Bufková, Ph.D.

The aim of this chapter is to briefly describe the principles of management in various types of natural, predominantly non-forest habitats (**Annex 13**) in river catchments with the presence of freshwater pearl mussel (FPM). This mainly concerns the following Sites of Community Importance (SCIs): Bystřina – Lužní potok (CZ0413177), Šumava (CZ0314024), Horní Malše (CZ0314022)

In adittion to meadow management, the chapter also includes a section on targeted composting, which has been practised in some areas for many years. Compost substrate is taken back to selected plots and is one of the ways to improve the nutrient content of FPM diet (for more details see **Chapter 5**).

The meadow management recommendations described below can be used not only by nature conservation authorities to establish appropriate management, but also by land users managing corresponding protected areas.

These measures are based on the state of habitats captured during a repeated field survey carried out in 2015 (https://aopkcr.maps.arcgis.com/). The Habitat Catalogue of the Czech Republic (HRCZ) (Chytrý et al. 2010) is routinely used in conservation and botanical practice; it details the characteristics of individual habitat types, their codes, representation of dominant and diagnostic plant species in communities, and maps of habitat distribution in Czechia. This chapter therefore describes the management of selected habitat types following the aforementioned catalogue (Chytrý et al. 2010).

9.1 FPM and plant cover

Current major problems in SCIs in which FPM is the object of protection are its reproduction and growth of its youngest developmental stages (**Chapter 3**). It is obvious that these problems are caused by several simultaneously operating factors, one of which is unsuitable vegetation on land adjacent to water courses with occurrence of this bivalve. Especially detrimental is the shading of floodplain and spring areas, which causes insufficient water heating for reproduction of the species. Another problem is posed by insufficient food resources, as already mentioned in **Chapter 5**.

Both problems have a fairly obvious cause residing in changes of vegetation cover that have occurred at all sites of interest over recent decades (Bílý et al. 2008). As a result of these changes in vegetation, and many other environmental factors, only populations of old, adult pearl mussels that do not reproduce spontaneously are known from these localities (Simon et al. 2015). Based on the age structure of today's FPM populations, it is clear that natural reproduction probably took place regularly until the mid-20th century, when fundamental changes in landscape structure and vegetation began to gradually occur.

For the purpose of analysing landscape-level changes of a past period, it is not useful to compare differences between actual and potential (Neuhäuslová et al. 1998) or reconstructed vegetation (Mikyška 1968). The reason is that the present-day distribution of FPM is strictly associated with habitats where fundamental vegetation changes took place during mediaeval colonization (see FPM distribution map Simon et al. 2015, or **Chapter 8**). No direct underlying sources are available for the analysis of vegetation cover during the period when spontaneous reproduction of the bivalve occurred.

Therefore, the best, albeit indirect, source of information on the structure of vegetation is offered by historical orthophotomaps from the mid-20th century (https://geoportal.gov.cz/). Orthophotomaps for the area of today's SCIs are from 1952. This means they were acquired soon after major demographic changes and in the period preceding the main changes that accompanied the collectivisation of agriculture after communist government started in 1948. Both spontaneous and anthropogenic successional changes in vegetation at the landscape level are therefore only recorded in their early stages.

It should be noted that analysis of historical orthophotomaps does not allow for a more accurate habitat assessment of the sites under study, only a general evaluation at a coarser (landscape) level. It nevertheless seems that such an analysis might provide answers to at least some questions concerning ways to improve the status of FPM populations.

9.2 Orthophotomaps and historical changes in vegetation development

All the SCIs under study are located in areas formerly inhabited by the Bohemian German-speaking population, which was expelled in 1945 after the end of World War II. Two of the study areas lie close to the state border, which is partly delineated by streams with FPM. The abrupt drop in settlement density has brought about remarkable changes to the agricultural use of the landscapes of these SCIs and the landscapes themselves. Non-forest land ceased to be used altogether (see **Chapter 8**). The structure of the landscape has changed gradually and the share of forest vegetation has increased remarkably. At present, the vegetation can be categorized mainly as stands of naturally regenerated woody species (non-natural habitat type X12[1] – for key of vegetation codes see **Annex 13**), even though there are also alder carrs along water courses and in spring areas (Habitats Directive priority habitat types L2.1 and L2.2). Today, some previously forest-free areas have the character of forests, but mostly with non-native tree species (non-natural habitat type X9A).

Analysis of forest-free areas does not allow a detailed assessment or comparison of past and current data. On orthophotomaps it is possible to more or less reliably distinguish fields from permanent grasslands, which, however, include different types of grassland vegetation. With grassland vegetation, it is possible to assess, at least in part, whether it grows in xeric or mesic stands or in stands strongly influenced by water. Based on a comparison with the current state, it can be assumed that the Habitats Directive habitats on xeric as well as mesic sites were the following: Mesic *Arrhenatherum* meadows (habitat type T1.1), Submontane and montane *Nardus* grasslands (T2.3) and Secondary submontane and montane heaths (T8.2), the non-Habitats Directive habitat type of *Cynosurus* pastures (T1.3), partly probably also the Habitats Directive habitat type of intermittently wet *Molinia* meadows (T1.9) and the non-Habitats Directive natural habitat types of alluvial *Alopecurus* meadows (T1.4) and wet *Cirsium* meadows (T1.5). On the other hand, wetland habitats were mostly the following Habitats Directive habitats: acidic moss-rich fens (R2.2), transitional mires (R2.3) and wet *Filipendula* grasslands (T1.6), and partly probably also intermittently wet *Molinia* meadows (T1 9) and the non-Habitats Directive natural habitat types of meadow springs without tufa formation (R1.2, on a small-scale), alluvial *Alopecurus* meadows (T1.4) and wet *Cirsium* meadows (T1.5). One specific structure, relatively easily distinguishable on orthophotomaps, are Habitats Directive raised bog habitat types: open raised bogs (R3.1) and degraded raised bogs (R3.4), which only occur at sites along the upper section of

[1] Names and codes of habitats are used in accordance with the HRCZ (Chytrý et al. 2010).

the river Vltava in Šumava SCI. For the reasons above, an analysis of the representation of individual non-forest habitats in orthophotomaps was not carried out for this study.

Orthophotomaps clearly show that groups of habitats were characterized by pronounced mosaic blending (for historical orthophotomaps of Lužní potok stream catchment see Bílý, 2008). Extensive technical measures (especially shallow drainage channels) probably existed across rather vast areas at that time. The mosaic of springs, wet and peaty meadows connected by small streams, as well as shallow drainage channels, were probably crucial to ensuring sufficient food supply for young stages of FPM. They ensured the formation of detritus and its transport to the main stream, in which the mussels developed. The significance of small streams as 'food rills' is described in more detail in **Chapter 5**.

After the continuity of private agricultural management was interrupted, some non-forest habitats were spontaneously overgrown by naturally regenerated stands of woody species, and some developed into wetlands that cannot be managed. Such wetlands have a relatively stable hydrological regime and occur predominantly along water courses with surviving FPM populations. Today they harbour, for example, habitats of Habitats Directive types of wet *Filipendula* grasslands (habitat type T1.6) and non-Habitats Directive natural habitat types such as reed beds of eutrophic still waters (M1.1), tall-sedge beds (M1.7), and riverine reed vegetation (M1.4), or degradation stages of the habitat type of wet *Cirsium* meadows (T1.5). Moreover, habitats of these types are sensitive to anthropogenic nutrient input and tend to become ruderalized. In such cases they gradually transform into non-natural habitats, namely ruderal herbaceous vegetation outside settlements with stands valuable for nature conservation (habitat type X7A), or into other stands (X7B).

After the collectivization of agriculture, large expanses of formerly forest-free land were managed using intensive agricultural practices. Many wetland habitats were rashly drained by subsurface drainage systems or deep open ditches. Drainage systems built in the 1980s as a result of the application of the then valid law continue to have a major impact. Great expanses of wetlands were transformed into mesic habitats. Today they are mostly represented by, to a large degree, intensively managed meadows or pastures. The most predominant is the non-natural habitat type of intensively managed meadows (habitat type X5), at best degraded, largely altered types, classified as the low-quality Habitats Directive habitat type mesic *Arrhenatherum* meadows (T1.1). From the point of view of food resources, these areas currently have no significance for FPM.

Studies in Blanice SCI (Blažková & Hruška 1999) in the second half of the 20th century indicate an expansion of *Carex brizoides* (quaking grass sedge) as one of the fundamental problems. In the second half of the 20th century, this species evidently expanded to abandoned areas in all the SCIs of interest. At present, however, a major problem in many parts of these SCIs is the recent extremely intensive expansion of *Phalaris arundinacea* (reed canary grass). Both these species have a fundamental impact on the availability of suitable food resources, as both usually produce monodominant dense stands that are resilient to the establishment of other species. According to Blažková & Hruška (1999), detritus from fallows degraded by *Carex brizoides* is completely nutritionally unsuitable for juvenile FPM (for more on detritus, see **Chapter 5**). It can be assumed that the same also applies to *Phalaris arundinacea*. Given that the impact of *Phalaris arundinacea* is recent, its biomass calcium content (which is important for young pearl mussels) has yet to be studied.

The list of changes undergone by the present landscape shows that FPM are facing the fatal problem of the availability of food resources. Drainage, which has fundamentally shaped today's hydrological regime, has destroyed the once fine mosaic of overlapping natural habitats. The result is a simplified habitat structure with sharp boundaries between mesic and wetland stands. This has decreased to the bare minimum the number of places with good food sources that are suitably **connected with the environment inhabited by pearl mussels**.

9.3 Characteristics of habitats in individual SCIs

9.3.1 Bystřina – Lužní potok SCI

In the second half of the 20th century, the catchment of Bystřina – Lužní potok SCI was subjected to substantial afforestation. Part of it has also been affected by deep drainage. Existing suitable food sources for FPM are limited in area. They are represented by a complex of grassland habitats near the rearing and reproductive feature by the Lužní potok stream (LRRF) and also by the Bystřina stream, the Brodivý potok stream, and others. A small part of the meadows in these parts of the floodplain is mown periodically (**Fig. 114**). Here, food rills have been restored or newly created, which positively influence the population of FPM and the habitats themselves. The remaining parts of the floodplain of the Lužní potok stream and other streams are becoming overgrown, predominantly by *Filipendula ulmaria* (meadowsweet; **Fig. 115**). Detritus from the litter of *Filipendula ulmaria* and from its roots does not influence young mussels positively (Blažková & Hruška 1999). On the German bank of the Lužní potok stream, meadows are mown to a much greater extent. However, some meadows on the floodplain also lie fallow. In the past, intermittently wet *Molinia* meadows (habitat type T1.9) were more widespread on both the Czech and German sides of the border (**Fig. 116**). Because of non-management, however, these are gradually turning into wet *Filipendula* grasslands (T1.6), or they are also being taken over by *Phalaris arundinacea*. A more detailed description of the SAC is provided on the NCA CR website and in the Summary of Recommended Measures (SRM) for Bystřina – Lužní potok stream SCI (Fiala et al. 2015).

Fig. 114. Managed meadows, Lužní potok stream floodplain.

Fig. 115. Unmanaged meadows overgrowing with *Filipendula ulmaria*, Lužní potok stream floodplain.

Fig. 116. Intermittently wet *Molinia* meadows (T1.9), Lužní potok stream floodplain.

9.3.2. Vltavský luh NM – Šumava SCI

In the vast Šumava SCI, the occurrence of FPM is concentrated in three places: the Vltava River within **Vltavský luh NM** (a 10 km section upstream from the backwater of Lipno reservoir), the **Blanice River** within Blanice National Natural Monument (NNM) and Prameniště Blanice NNM, and in the **upper part of the Zlatý potok stream catchment.** For the sake of clarity, these three territories are dealt with in separate sections, even though they all lie within Šumava SCI. To avoid a misunderstanding, Blanice SCI and Zlatý potok stream SCI in the Šumava region are both located downstream (and outside Šumava SCI) and are not among the priority localities with FPM; only small numbers of FPM survive there. Most places with the occurrence of the mussel in the **Vltavský luh** marshland are designated as core zone of Šumava National Park.

Alluvial meadows adjacent to the water course were in effect not managed in the second half of the 20th century and had lain fallow for a long time. At present, previously drained waterlogged areas are undergoing succession by woody species, and this also applies to bogs with a disturbed water regime. Large, unmanaged areas in more fertile depressions are becoming wettened and are growing over with monotonous wetland vegetation in which *Phalaris arundinacea* is spreading (**Fig. 117**); *Carex buekii* (Banat sedge) and *Spiraea salicifolia* (willow-leaved bridewort) have begun to spread in recent years (**Fig. 118**). Peaty meadows are only preserved in small parts of the area meaning both non-calcareous moss peat bogs (habitat type R2.2) and transitional mires (R2.3). On the Vltava floodplain there are also fragments of alluvial *Alopecurus* meadows (habitat type T1.4; **Fig. 119**) and intermittently wet *Molinia* meadows (T1.9). In some places, meadows are being mown or other suitable management is under preparation.

Marginal parts of the core zone of the National Park and adjacent areas have often been afflicted by large-scale drainage. Still, a large number of suitable food sources remain in the area and they even seem to be suitably linked with the river bed itself. One probable advantage of the Vltava floodplain is that detritus from water macrophytes present within the river itself is a good source of

Fig. 117. Site overgrowing with *Phalaris arundinacea*, Vltavský luh marshland.

Fig. 118. Spreading *Spiraea salicifolia*, Vltavský luh marshland.

Fig. 119. The last remnants of alluvial *Alopecurus* meadows, Vltavský luh marshland west of the village of Chlum. The area is being managed.

nutrition for young pearl mussels. Furthermore, a number of measures have been implemented over the past decade to promote the recovery of suitable habitats. On the floodplain, mowing of different types of alluvial and peaty meadows has been reinstated on 55 ha of land. A suitable water regime is gradually being restored. Straightened deep channels of regulated water courses are being replaced by shallow, naturally meandering river beds (5 km of natural river beds have been restored) and the intensive drainage network is being dismantled. On the left bank slopes above the floodplain, intensive agricultural management ended in the 1990s, and fertilized arable fields were replaced with mown meadows and extensive pastures. However, the main problems in the Vltavský luh marshland are probably the changing climate and a lack of fish hosts (*Salmo trutta* m. *fario*). For more information on fish stocks, see **Chapter 5**.

9.3.3 Šumava SCI – NNM Blanice

The fundamental problem of Blanice NNM is the small number of suitable food sources for FPM, which are being partly overgrown by trees and partly destroyed by drainage. In a part of the area, the river Blanice is lined by a practically unmanageable floodplain with deep drainage ditches, which is now being intensively overgrown by expansive species. This mainly involves *Carex brizoides* and *Phalaris arundinacea* (**Fig. 120**). In contrast, crops are being grown in several places, which also cause food shortages for pearl mussels. There are very few species-rich mesic meadows on the Blanice floodplain; managed meadows and intensive pastures predominate. Only the last remnants of peaty meadows are preserved on the floodplain (**Fig. 121**); in many places, however, they are being overgrown, are degrading, or are turning into vegetation of different types. Besides existing rearing and reproductive features (RRFs), there are very few places in the NNM where it would be possible to improve the environment for FPM by appropriate modifications. Such measures, however, require significant financial resources and their outcome is uncertain.

Fig. 120. Banks and gravel alluvia overgrowing with *Phalaris arundinacea*, Blanice River, southeast of the village of Spálenec.

9.3.4 Šumava SCI – Zlatý potok stream

This is a relatively well-preserved catchment with a mosaic vegetation of meadows, spring areas, spontaneously established vegetation growth, and forest stands. Many meadows in the area have not been managed for a long time. However, the Zlatý potok stream floodplain has not been spared areal eutrophication, and many of the unmanaged meadows are being overgrown by *Filipendula ulmaria*, *Phalaris arundinacea*, and *Carex buekii*. Even so, various types of meadows remain scattered on the floodplain. However, because of changes in management, it is impossible to classify them phytocoenologically with any certainty. These changes have been caused by the modification of hydrological conditions, fertilization, sowing of agricultural grasses, and the replacement of manual labour with machinery. The meadows in the area are transitional between alluvial *Alopecurus* meadows, wet *Cirsium* meadows, and meadows of mesic types (**Fig. 122**).

One of the most important places where FPM thrive is the rearing and reproductive feature at Zlatý potok stream (ZRRF). The main component of the reproductive environment of the mussel in the proposed Zlatý potok stream NNM is the spring area near the village of Skříněřov, which requires thorough targeted restoration. Until the impaired flow functions of the Zlatý potok stream are restored, the ZRRF provides pearl mussels with a suitable environment with auxiliary food management. However, the optimal reproduction environment for the mussel is terminated by a tributary, the eutrophicated Tisovka stream, immediately below ZRRF.

9.3.5 Horní Malše SCI

The border section of the Malše River (**Fig. 123**) (on the Bohemian side) is mainly lined with unmanaged wetlands whose potential for improving the food situation of FPM is unclear. Some suitable areas have been overgrown by spontaneously established woody species. On the Bohemian side of the border, unmanaged meadows are gradually changing, *Phalaris arundinacea* being predominant (**Fig. 124**). On the Austrian side, in contrast, most of the land is being used agriculturally almost right up to the river. It is more or less obvious that the decisive factor for the pearl mussel is not so much the lack of nutrition coming from meadows, but rather water temperature, erosion and, lower downstream, also eutrophication. A more detailed description of the SCI can be found on the NCA CR website and in the SRM for the SCI (Weiter & Indra 2015).

Fig. 121. Peaty meadows, Blanice River floodplain, south-east of the village of Spálenec.

Fig. 122. Regularly mown wet *Cirsium* meadows and peaty meadows, the village of Miletínky.

Fig. 123. Malše River floodplain with bank vegetation.

9.4 Management of meadow habitats and alluvial forests

Management measures are proposed for all natural non-forest habitats and for riverine and spring alder carrs, as well as for the degraded phases of these natural habitats at sites located in catchments with the occurrence of the FPM (the Lužní potok, and Bystřina streams, and the rivers Blanice, Malše, and upper Vltava). For forest habitats surrounding streams (i.e., floodplain forests), only basic principles of management are proposed below. The proposed principles of management comply with the management plans of individual sites and also with the SRM for SCIs. The management of the habitats selected is organized according to habitat types following the HRCZ (Chytrý et al. 2010).

9.4.1 V4A Macrophyte vegetation of water streams

Habitats Directive: 3260 Water courses of plain to montane levels with Ranunculion fluitantis and Callitricho-Batrachion vegetation

The Teplá Vltava River, the Lužní potok and Bystřina streams (**Fig. 125, 126**), and unregulated sections of the upper Malše river are very rich in aquatic macrophytes. More than ten species of aquatic macrophytes occur in the Vltava; some of the most important are alternate *Myriophyllum alterniflorum* and *Potamogeton alpinus* (alpine pondweed; **Fig. 127**). Common species occurring in such habitats are *Callitriche hamulata* (intermediate water starwort), *Sparganium emersum* (European bur-reed), and *Batrachium peltatum* (pond water-crowfoot). In some places, river arms or side pools are infested with *Elodea canadensis* (Canadian pondweed), whose populations, however, do not thrive in upper oligotrophic stretches of rivers. The Lužní potok and Bystřina streams are characterized by the occurrence of *Potamogeton polygonifolius* (bog pondweed; **Fig. 128**); *Potamogeton alpinus* is also locally

Fig. 124. Overgrowing bank stands with *Phalaris arundinacea*, Malše River.

Fig. 125. Water courses rich in water macrophytes, Teplá Vltava River.

present and *Callitriche hamulata* is abundant. *Callitriche hamulata* and *Batrachium peltatum* are also highly abundant in some sections of the upper Malše River; *Fontinalis antipyretica* (antifever fontinalis moss) is also relatively frequent. In the river Blanice, the incidence of macrophytes is limited to bryophytes and *Callitriche hamulata*.

The richness of aquatic macrophytes, both in terms of overall cover and species diversity, has a positive effect on water detritus. The quality of such habitats is therefore of great importance for the nutrition of FPM. Active management is not carried out. Nevertheless, it is important to regulate the use of the Teplá Vltava River for water tourism (canoeing) to a level acceptable from the point of view of the protection of the local FPM population and the decline of macrophyte-rich communities (Kladivová & Simon 2009).

Fig. 126. Lužní potok stream.

Fig. 127. *Potamogeton alpinus* in Vltava River.

Fig. 128. *Potamogeton polygonifolius* in Lužní potok stream.

9.4.2 M1 Reeds and tall-sedge beds

M1.1 Reed beds of eutrophic still waters

These are stands of *Phragmites australis* (common reed) or *Typha latifolia* (broadleaf cattail) in oxbow lakes or ponds (**Fig. 129**) in different phases of infilling. Terrestrial reed beds outside oxbows or ponds are part of the X7A category of unnatural habitats. As regards food resources, this habitat type has no significance for FPM.

If no dredging of river arms or ponds is carried out to support aquatic macrophyte vegetation, amphibians, and other animals, there is no need for any management measures.

M1.4 Riverine reed vegetation

This category comprises vegetation dominated by *Phalaris arundinacea* on river banks (non-natural habitat type X7A on bulldozed and otherwise altered banks) and *Carex buekii*. These stands along rivers are often extensive and difficult to pass through. Vegetation with *Carex buekii* is especially abundant along the banks of the river Vltava (**Fig. 130**). On the banks of major water courses, these stands are probably semi-natural (surrogate) communities following deforestation. However, in the past such communities were probably of a linear, not areal, character and were apparently never utilized as meadows. In recent years, vegetation with *Phalaris arundinacea* has been spreading along the Lužní potok stream, the rivers Malše and Vltava, and the Zlatý potok stream (**Fig. 131**). From the nutritional perspective, habitats of this type have practically no significance for FPM.

No management is needed in these stands, and it would probably not make sense to commence any management there. This would require a great deal of human effort, a huge amount of funds, and the result would certainly not be commensurate with the money spent.

Fig. 129. Stands with *Phragmites australis* in an infilled pool.

Fig. 130. Stands with *Carex buekii*, Vltavský luh marshland.

Fig. 131. Stands with *Phalaris arundinacea*, Vltavský luh marshland.

M1.5 Reed vegetation of streams

Vegetation with *Glyceria* sp. (sweet-grasses) and *Veronica* sp. (speedwells) occur less frequently along smaller streams and on clayey banks of river branches (**Fig. 132**). Sometimes *Mentha* sp. (mint species) are present as well as other riverbank plants. Generally, this type of vegetation is less abundant and also largely overlooked because of its small-scale distribution. It cannot be ruled out that detritus produced in habitats of this type is important for FPM (species with favourable litterfall are present in this vegetation), but there is no relevant information on the actual representation of these stands in and around pearl mussel sites. This vegetation does not require any management.

M1.7 Tall sedge beds

In areas with FPM, vegetation of this type can occur by oxbow lakes and in very wet places in depressions in meadows, occasionally in frequently flooded meadows, or in places with stagnating rainwater. In their depauperate form (poor species composition), meadows of this type often represent successional stages of clogged drainage channels. The dominant species of this vegetation in these areas include *Carex acuta* (tufted sedge), *C. buekii*, *C. lasiocarpa* (slender sedge), *C. rostrata* (bottle sedge; **Fig. 133**), *C. vesicaria* (bladder sedge), and sometimes other sedge species. In terms of food resources, this habitat type is likely to be of practically no importance for the pearl mussel because detritus from tall sedges is slow to decompose and contains few accessible nutrients.

Such stands usually do not require any management, the exception being sedge stands which are beginning to spread in peaty meadows and wet *Cirsium* meadows. The management of such stands is described in the corresponding sections on these habitat types.

Fig. 132. Stands of *Veronica beccabunga* in a small stream.

9.4.3 R1 Springs

R1.2 Meadow springs without tufa formation

This habitat type occurs in areas with profuse or permanent springs in meadows, peat bogs, near streams, and, rarely, in other types of non-forest vegetation. The species composition may vary; the most valuable areas harbour vegetation with *Montia fontana* (water blinks; **Fig. 134**). More common species of springs include *Stellaria alsine* (bog stitchwort), *Cardamine amara*, *Epilobium obscurum* (dwarf willowherb), *Glyceria fluitans* (floating sweet-grass), *Chaerophyllum hirsutum* (hairy chervil; **Fig. 135**), and *Veronica beccabunga* (European speedwell). Detritus formed in habitats of this type is very important in terms of yield.

No management is required in spring areas that are not being overgrown. Springs that start overgrowing should be mown at least once every two to three years. Excessive overgrowth can be identified by the fact that there are almost no open areas with a clear water surface. Usually, once the spring is mown, the activity has to be repeated regularly. Typical manifestations of overgrowing include increased biomass of grasses or herbs. An increased presence of *Alopecurus pratensis*, *Poa trivialis* (rough-stalked meadow-grass) or the *Holcus lanatus* (meadow soft grass), as well as of other grass or herb species, indicates degradation.

In meadow springs that are overgrowing with trees or shrubs, it is advisable to carefully remove naturally regenerating trees and shrubs (preferably when still young, i.e., up until about five years of age). In meadows that have been overgrown for a long time, it is necessary to gradually carry out the removal of woody species. They should never be removed all at once because it would increase the risk of the spring area being colonized by other species that would accelerate the degradation process. The succession of springs can be greatly influenced, for example, by the occurrence

Fig. 133. Stands with *Carex rostrata* on the banks of an oxbow lake, Vltavský luh marshland.

Fig. 134. Spring with *Montia* sp. (water chickweed), Šumava Mountains.

Fig. 135. Spring with *Chaerophyllum hirsutum*, near the village of Spálenec.

of shrubby willows (*Salix aurita* – eared willow and *S. cinerea* – grey willow). During a management activity, willows may be pruned carefully without destroying the whole bush, reducing the risk of further degradation.

Opening up of meadow springs is very important from the point of view of FPM, especially because it helps maintain a favourable temperature regime (see **Chapters 5** and **10**). Water temperatures increase in springs and in small streams; in the main water course they are usually stable.

In spring areas within grasslands that are regularly grazed, it is essential to enclose all springs in such a way that animals cannot access them, in order to prevent degradation by trampling and faeces. Excessive trampling of springs leads to the retreat of most meadow species and the predominance of *Juncus effusus* (soft rush; **Fig. 136**) and *J. conglomeratus* (compact rush). Their predominance is another negative indication of the habitat type of springs, whose indicator species usually disappear as a result of extensive disturbance.

Many meadow springs have been drained and the outflow of water has been increased and accelerated by surface channels. Thanks to abundant saturation with water, even such damaged springs function well and harbour typical vegetation, albeit on a reduced area and in depauperate form. In such places, it makes sense to block or backfill deep drainage channels, while restoring natural shallow rills from the springs to the water course. Restoration of drained spring areas is usually necessary because degradation tends to be progressive, even many years after drainage, and remnants of suitable herbal vegetation may disappear completely over time.

Fig. 136. Spring (helocrene), Spálenecký potok stream, springtime. Stands of *Juncus effusus* are spreading as a result of soil disturbance, probably the long-term effects of wild game.

R1.4 Forest springs without tufa formation

Bryophytes sometimes predominate in forest spring areas on hummus-rich, muddy, and also rocky substrates. More structured vegetation develops only in areas with more widespread flooding (**Fig. 137**). In some cases, the species composition of forest spring habitats is not too different from that of wetland forest habitats. Forest springs are usually not as sensitive to overgrowth unless there is significant eutrophication or artificial drainage. However, degradation of springs may also occur as a result of abrupt opening of the canopy (e.g., by logging). **Detritus formed in this habitat is important for** FPM**.**

It is never permissible to interfere with the natural water regime of spring areas in catchments with FPM or to cause any damage to the water regime of springs. Forest springs near water courses (up to ca. 500 m from the stream) should have their vegetation very delicately (partly) opened up to facilitate the washing away of high-quality detritus, which serves as food for young pearl mussels. This opening up should be done in cases where the spring is connected to the main flow or when targeted restoration is carried out at the same time, for example the restoration of a spring channel (see **Chapter 10**) that facilitates the connection between the spring and the water course, which promotes the influx of detritus into the main stream. The opening of the vegetation should be carried out sensitively and gradually to avoid disturbance and degradation of the spring area. If the spring is not connected to the main flow, or is far enough away from it, no management should be performed. As with meadow springs, the main problem for forest springs are surface drainage channels. The principles of restoring the water regime of drained forest springs to support high-quality herb vegetation producing suitable detritus are basically the same as with the previous habitat type.

Fig. 137. Herb layer of a woodland spring with *Chaerophyllum hirsutum*, *Cardamine amara*, and *Chrysosplenium alternifolium*.

9.4.4 R2 Fens and transitional mires

Peaty meadows are sometimes highly diverse and are one of the very frequent habitat types in catchments with FPM. Most common in the landscape are peaty meadows with different types and degrees of succession and signs of degradation. Peaty meadows that are not being overgrown are rather exceptional in today's landscape. The main causes are three degradation factors: drying, eutrophication, and excessive waterlogging. One of the first signs of degradation is usually the replacement of species in the mossy layer and the retreat of the most sensitive, competitively weak species. A further sign of degradation is the general retreat of the moss layer and the spread of certain dominant angiosperm species. A sign of greater degradation is increased waterlogging and the spreading of more aggressive, often woody, species. Peaty meadows may also gradually turn into wet *Cirsium* meadows, which with ongoing successional changes even may be transformed into habitats of non-natural type X7A. The crucial negative effect is damage to the water regime, which has happened in virtually all river catchments. Degradation, however, manifests itself gradually and in different ways, which is related to the overall hydrological regime and also to the sources of nutrients that are flushed into water courses.

Some types of fens, especially transitional mires, do not require any management. Others used to be occasionally mown in the past. With degraded communities, it is necessary to decide whether it is possible to gradually reconstruct the original state, at least in part, and whether the effect of the funds and human effort invested is appropriate for the state of the vegetation. It is not always possible to reliably estimate how different types of vegetation will respond to management. Sometimes it is enough to remove spontaneously established woody species, but other times it is necessary to carry out more effective mowing, usually once a year. Only in some cases of expanding strong dominants (e.g. *Molinia caerulea* – purple moor-grass) is more vigorous mowing desirable, at least initially twice a year. In many cases, today's degraded sites have been drained by deep surface channels. In such cases it is usually necessary to fill in or block deep channels so that the resulting state after restoration corresponds to the water regime of a maintained meadows with its former system of shallow channels. Without the elimination of deep drainage channels, it is usually impossible to stop the progressive degradation of disturbed sites, even by recommencing appropriate management (i.e., mowing). However, such a restorative intervention may lead to completely different types of wetland vegetation. It is not easy to predict the result. With peaty meadows, site accessible for machinery is crucial, as is how grass biomass can be disposed of. It is certainly not a good solution to dispose of this biomass in the vicinity of the site, that is, to store or spread mown material in its immediate surroundings. One possible substitute solution is to construct a natural compost pile, but this is highly laborious and would increase the cost of the management. Moreover, in the highly rugged environment of the Vltava floodplain, the placement of compost piles is challenging, considering the intricate mosaic of oligotrophic habitats, including nutrient-poor pools harbouring rare communities and species. A limiting factor in this area is regular flooding, which can carry away any composted material to a different site and thus cause undesirable local eutrophication.

R2.2 Acidic moss-rich fens

Habitats Directive: 7140 Transition mires and quaking bogs

This category comprises low to medium-tall sedge stands whose species diversity may be low, although stands on more alkaline substrates may be significantly species-rich. In stands little affected by human activity there are a number of endangered and protected plant species. Detritus formed in species-rich stands, where other species occur besides smaller sedges, is important for FPM. However, degradation involving species depauperation and a prevalence of *Cyperaceae* family species (mainly club-rushes and sedges) decreases the trophic level considerably.

Signs of degradation are highly diverse. The most common signs of degradation include succession by overgrowth. This usually occurs when the content of soil nutrients is elevated by eutrophication as a result of atmospheric deposition or runoff from agricultural crops, and it is often accelerated on fallow areas. Other causes of degradation include disturbance by cattle grazing and machinery during mowing, drying out or, in contrast, gradual waterlogging, which is often caused by a rise in the water level as a result of the clogging of former shallow drainage channels. Peaty meadows may overgrow with a number of more aggressive and competitively stronger species. Frequent indicators of degradation include, for example, *Scirpus sylvaticus* (wood club-rush) or *Filipendula ulmaria*; excessive waterlogging leads to the spreading of tall sedge species. In some areas, spreading into various types of peaty meadows, usually those drained in the past, are *Carex brizoides*, *Deschampsia caespitosa* (tufted hairgrass) and *Phalaris arundinacea*. In some areas, spreading in this way are also *Alopecurus pratensis*, *Galeopsis bifida* (bifid hemp-nettle), *Senecio* sp. (ragworts and groundsels) as well as other species. Excessively grazed peaty meadows damaged by trampling and elevated nutrient levels are usually overgrowing with spreading rushes (e.g., *Juncus effusus*). Peaty meadows are also under threat from being overgrown by naturally regenerating woody species, mainly *Picea abies* (Norway spruce), alders (*Alnus glutinosa* and *A. incana*), and shrubby willows (especially *Salix cinerea* and *S. aurita*).

There is no need to regularly mow peaty meadows (**Fig. 138**) without signs of degradation, but their favourable state can change suddenly. For these reasons, it is advisable to constantly monitor the development of vegetation at key locations. Once peaty meadows start to be overgrown as part of natural succession, and signs of their degradation begin to show (including the ones mentioned above), it is time to subject them to regular management. Mowing once a year or every second year

Fig. 138. Peaty meadows (R2.2) in a spring area, Sněžný potok stream (left-hand tributary of the Blanice River, below the village of Spálenec), in a mosaic with vegetation of wet *Cirsium* meadows (T1.5a).

is usually sufficient, normally in late summer or at the turn of July and August. It all depends on the current growing season and the exact cause of degradation. If the season is wetter, meadows of this type can be mown earlier; otherwise, especially if the season is drier, it is advisable to shift the mowing period to the very end of summer. It is important to carry out mowing in the drier part of the season and it is always necessary to thoroughly rake out the cut biomass, which must be taken away from site. Often it is necessary to only mow peaty meadows with a brushcutter (or scythe) or a small hand-guided mower. Peaty meadows mown by a tractor undergo substantial degradation. Rushes spread, especially in wheel ruts, and are difficult to get rid of. Repeatedly strongly compressing the layers of peat destroys water-retaining pores, which is why valuable hygrophilous species often disappear from mown plots.

Older spontaneously established woody species, if there are any, should only be cut when really necessary. It is better to pull out or cut young trees or shrubs. It is better to leave older trees on site. It is almost never appropriate to cut down all spontaneously established trees and shrubs at once. Instead, they have to be removed gradually. This is important not only in terms of subsequent degradation of vegetation, but also for supporting insects and other animals.

It is always advisable to leave at least some parts of peaty meadows without mowing in order to preserve the mosaic of mown and unmown areas in the landscape. To avoid damaging adjacent stands, peaty meadows intended to be mown should be selected according to their accessibility for machinery.

R2.3 Transitional mires

Habitats Directive: 7140 Transition mires and quaking bogs

This category comprises hillside or valley mineralotrophic peat moss stands which are usually poor in species and are characterized by a high cover of moss layer (**Fig. 139**). Often they are in close contact with non-calcareous moss fens or with bogs. Similar to acidic moss-rich fens, types richer in species with a diversified structure are the most important for FPM; their trophic level is significantly decreased when diversity is depauperated and sedges or rushes predominate. Meadows influenced by long-term damage to the water regime exhibit a variety of degradation processes. These include eutrophication, which, together with drainage, causes the transformation of transitional peat bogs into vegetation of a different type. One conspicuous manifestation of degradation in transitional peat bogs is the retreat of the moss layer (both in quantity and in quality). Succession after drainage results in more dry-loving types of vegetation, in which grasses (*Poaceae*) predominate over time. In the final phase, such a course of succession can lead to waterlogged, degraded mesic to anthropically eutrophic stands. Signs of their degradation include the spread of species from *Cirsium* meadows, for example *Caltha palustris* (marsh-marigold), *Cirsium oleraceum* (cabbage thistle) and *C. palustre* (marsh thistle), and *Chaerophyllum hirsutum*. In such vegetation, the moss layer recedes, meadow species spread massively, and entire stands may turn into highly waterlogged wet *Cirsium* meadows. An advanced negative phenomenon is overgrowing by intensely spreading grasses, for example *Holcus mollis* (creeping soft grass), *Carex brizoides*, *Filipendula ulmaria* and other species. Degradation of a different kind occurs in places with excess water, where unmanaged permanently flooded patches often develop into monodominant stands of *Carex rostrata* , usually with only a minimum of sphagnum mosses. In some localities that are left fallow and are well fed with water, the influx of nutrients promotes the growth of acidophilic tall-growing *Sphagnum* species, which changes the pH of the environment. As a result, the species richness of vascular plants and bryophytes gradually decreases and only a few species prevail, often for example *Carex rostrata*. In many places, both aforesaid types of degradation tend to be exacerbated by eutrophication caused by excessive nutrient runoff. Degradation may manifest itself differently in different areas. Over the next two years, new influences and new hitherto unknown types of degradation may come into play.

The vegetation of transitional mires has probably never been mown or it was mown highly irregularly. Better preserved types or types with very slight signs of degradation should be left without management in the long term. In contrast, where signs of degradation are markedly significant, there is no point in carrying out any management for the protection of FPM.

Only in places where transitional mires are being strongly overgrown with naturally regenerating woody species (spruce, birch, and willows) is it always advisable to pull up or cut out young trees in particular (they can be pulled up manually in peat bogs). It is always better to retain older mature spruce or birch trees because cleared areas can potentially degrade. There is a risk of subsequent succession involving species that could expand throughout the site (any new activity, including felling trees, can cause negative phenomena). Spreading willow shrubs should be trimmed gradually without destroying them immediately, as this would then leave open areas for the spread of competitively stronger species.

9.4.5 T1 Meadows and pastures

T1.1 Mesic Arrhenatherum meadows

Habitats Directive: 6510 Extensive hay meadows of the plain to submontane levels (Arrhenatherion, Brachypodio-Centaureion nemoralis)

Mesic *Arrhenatherum* meadows (**Fig. 140**) are dominated by grasses. Herbs are well represented in their species-richer types. In the past, most of these meadows, many of which are former fields, were either established or supplemented by sowing commercial grass mixtures. High-quality, species-rich *Arrhenatherum* meadows are present only in very limited areas in the wider surroundings of FPM localities. If a habitat of this type is present, it is usually in some way altered or degraded. However, if natural habitats are still present in the vicinity, such depauperate types may be continuously enriched by species from the surroundings. **Detritus produced on mesic *Arrhenatherum* meadows (especially extensively managed, less productive types) is suitable for FPM, but its transfer to the aquatic environment poses a challenge.**

Arrhenatherum meadows used to be mown twice a year: in June and again in August. The appropriate method of management very much depends on the specific location and on the weather in the given year. Some nutrient-poorer types of mesic meadows can be mown only once a year, but the more productive types require mowing twice a year. The frequency of mowing also depends on the distribution of wet and dry weather in the given growing season. It is advisable to dry hay directly on the meadow, so that the soil bank remains supplied with seeds of meadow species. If mown grass biomass is removed early and hay is left to dry completely outside the meadow itself, seeds are taken away each year, which can also lead to species depletion. It is always necessary to mow the meadow all the way to the edges of roads, where ruderal species signalling increased eutrophication are usually present. The reason is the need to prevent the spread of these species not only into the surrounding area, but also into the grassland itself. Meadows of this type can exceptionally be fertilized every three to five years, but only with mature or composted manure.

Fig. 139. Fragment of a transitional mire, Blanice River floodplain, north of the village of Spálenec.

Fig. 140. Mown mesic, species-rich meadow, the village of Záhvozdí.

T1.2 Montane Trisetum meadows

Habitats Directive: 6520 Mountain hay meadows

In the Šumava Mountains, the habitat type of mown montane meadows is very rare (**Fig. 141**), currently more in the form of transitional types. In areas with FPM outside Šumava SCI, *Trisetum* meadows are not present. There are nutrient-rich mesic meadows of the submontane to montane belt with the occurrence of montane species, which include, for example, *Geranium sylvaticum* (wood cranesbill), *Poa chaixii* (broad-leaved meadow-grass), *Trisetum flavescens* (yellow oatgrass), and the *Centaurea pseudophrygia (*knapweed). Their species composition has changed considerably as a result of increased trophic level. Precisely such stands belong to non-differentiated types tending to be transient between habitat types T1.2, T2.3B, and T1.1, which are related to the association *Poa-Trisetetum flavescentis* variant *Hypericum maculatum* (Chytrý 2007) and are, moreover, probably closer to habitat type T1.1. Detritus produced in stands of montane *Trisetum* meadows is possibly relatively suitable for the pearl mussel. However, as with mesic *Arrhenatherum* meadows, there is the problem of its transfer to the aquatic environment.

In catchments with FPM, montane *Trisetum* meadows are represented only marginally and in small areas (they may even be completely absent) and their management does not have a major effect on the mussel.

Montane *Trisetum* meadows degrade when they are left fallow. They overgrow rapidly and degraded stands are difficult to restore.

Mown mountain meadows were usually cut once a year, at the turn of June and July or by the end of July, depending on the type of vegetation season. In wet seasons they were mown earlier, in drier

Fig. 141. Montane *Trisetum* meadows, central Šumava Mountains.

seasons later. One mowing per year usually sufficed. In most cases meadows of this type were never grazed. The same is recommended for the present-day vegetation of mountain meadows. As with *Arrhenatherion* meadows, it is recommended to dry the hay on site. In exceptional cases, such meadows can also be fertilized at intervals of three to five years, but only with mature or composted manure.

T1.3 *Cynosurus* pastures

In the Šumava region, *Cynosurus* pastures are rare. Most of them are grazed grass and forb communities of a cultural character (**Fig. 142**). Their significance as a food source for FPM is probably marginal. A major problem with recent pastures is the fact that their species composition has usually been severely damaged by the sowing of commercial seed mixes. This has more or less eliminated low-productive species, which coped well with disturbance by trampling, examples being *Cynosurus cristatus* (crested dog's-tail) and so-called pasture weeds, for example stemless *Carlina acaulis* (carline thistle). *Cynosurus* pastures with a disturbed species composition degrade rapidly if they are grazed by an excessive amount of cattle every season, and also if the cattle stay overnight. This creates strongly eutrophicated patches colonized by ruderal species, which spread quickly to other parts of the pasture. The restoration of such eutrophic stretches of land is highly complex and often ineffective.

Cynosurus pastures used to be subjected to intensive grazing once a year, but only for less than three weeks and usually by a comparatively small number of cattle (1.5 livestock units (LU) per ha). However, it is often important to choose a suitable breed. In any case, it is better to select one that does not live in herds. Animals should not stay on pastures overnight. After grazing, it is advisable to leave the stand ungrazed for one to two seasons. During this period it is appropriate to mow the area at least once every season. Alternation of different grazers (i.e., horses, cattle, and sheep) is considered ideal.

Fig. 142. Cultivated grazed grassland, possibly a former habitat of *Cynosurus* pastures.

T1.4 Alluvial Alopecurus meadows

This category of habitats comprises wet alluvial meadows with *Alopecurus pratensis*, *Holcus lanatus*, and *Deschampsia cespitosa*. In the catchments of the rivers Blanice (**Fig. 143**) and Vltava (**Fig. 144**), *Deschampsia caespitosa* meadows apparently used to be a more common type of vegetation than in the other areas under study. The current situation, however, is strongly affected by the water regime, which has been damaged on a landscape scale. *Alopecurus* meadows tend to be poorer in species, especially along the Blanice River. However, preserved along the Teplá Vltava River are fragments with rather high species diversity. Nevertheless, the degradation of alluvial *Alopecurus* meadows is currently proceeding very quickly. Such meadows in the catchments of the Vltava River and the Zlatý potok stream are being overgrown by *Carex buekii*, *Phalaris arundinacea* and often also by *Filipendula ulmaria*; also, in the Blanice River catchment, mainly by *Phalaris arundinacea*. Currently, there are no *Alopecurus* meadows in the catchments of the Malše River and the Lužní potok stream. **The habitat type of alluvial *Alopecurus* meadows is of great importance for FPM in terms of trophic level.**

Nutrient-richer types of *Alopecurus* meadows with *Alopecurus* sp. (foxtail) and *Holcus* sp. (soft grass) should be mown once or twice a year. Stands with the *Deschampsia caespitosa* and stands with fewer species can be mown only once a year, at the turn of July and August. It is also advisable to dry hay directly on site and to transport away only already dried biomass to provide enough seeds from grasses and herbs for the soil seed bank. However, salvaging the last remains of preserved *Deschampsia caespitosa* meadows in the Vltava River and Blanice River catchments will require targeted management, the details of which remain to be worked out. This also entails the possible creation of a certain infrastructure, for example hay barns, from which biomass can be transported only after freezing, as was done in the past. In some places, mowing alone is sometimes inadequate, especially if the water regime has been severely damaged. Restoration of the water regime is highly problematic, and excessive eutrophication prevents the return of types of vegetation present fifty years ago. Unmown *Alopecurus* meadows are expected to completely disappear from the area within the next twenty years.

T1.5 Wet Cirsium meadows

In the past, wet *Cirsium* meadows were probably the most widespread vegetation unit of wet meadows in all the areas under consideration (**Fig. 145**). One consequence of this is their considerable variability. Such vegetation tends to be very rich in species, especially if it is managed in an appropriate way. However, species-rich types are no longer abundant in the landscape. Unmown stands are often species-poor and are usually characterized by a single markedly dominant plant species. With sufficient nutrient supply (especially alkaline nutrients), meadows that are not regularly mown may transform into *Filipendula* grasslands (Habitats Directive habitat type T1.6). In other cases (especially also in connection with more permanent waterlogging) degradation occurs as a result of the dominance of a single plant species, for example *Scirpus sylvaticus* or *Chaerophyllum hirsutum*. Spreading locally on *Cirsium* meadows that have long been fallow and possibly drained in the past are *Carex brizoides*, other tall sedge species, and sometimes also *Phalaris arundinacea*. **The habitat type of wet *Cirsium* meadows is of great importance for FPM in terms of trophic level, but only if the vegetation is varied and with high species diversity**. Degradation, which is often accompanied by a loss of species, in most cases leads to the deterioration of the nutrient status of the habitats.

Wet *Cirsium* meadows are usually mown twice a year, and hay is produced on site and transported away. It is not very efficient to mow severely degraded *Cirsium* meadows. The regeneration process takes a very long time, and the energy and resources spent are not proportional to the condition of

Fig. 143. Degraded alluvial *Alopecurus* meadows on gravel sediments, Blanice River floodplain.

Fig. 144. Alluvial *Alopecurus* meadows with *Holcus lanatus*, Vltava River floodplain.

areas with such management. Similarly, it is not worthwhile to commence management on extremely eutrophic wet *Cirsium* meadows. Intensive grazing of wet *Cirsium* meadows promotes their degradation. Disturbances are especially severe in places with spring outflows and lead to the spread of rushes (*Juncus effusus, J. conglomeratus*) or sometimes the *Poa trivialis* and other competitively strong species.

Carex brizoides (**Fig. 146**) usually colonizes damp habitats belonging to this habitat type (T1.5 W); if it expands into dry habitats, the vegetation is categorized among unnatural habitat types (X7A). The management of stands with this sedge as the dominant makes little sense, and they are better left without intervention. Neither the money spent nor the human effort are proportional to the effect of mowing or any other type of management. Not even grazing is suitable in these types of habitat, as cattle mostly avoid consuming *Carex brizoides*. In some cases, it is justified to mow these stands, but to suppress this strongly dominant sedge and to improve the species composition, slight fertilization with organic fertilizers is necessary. However, the regeneration process can take a very long time.

Management of heavily degraded stages of wet *Cirsium* meadows makes sense in situations where better-preserved types of wet *Cirsium* meadows recede to a minimum area and cease to fulfil their function for FPM. However, it is always necessary to obtain a local expert assessment of which stands should be managed, and which should not. In particular, it is important to consider the distance of the segment being reconstructed from a breeding and rearing feature, or from places along the main water course with the occurrence of FPM.

Fig. 145. Wet *Cirsium* meadows with dominant *Scirpus sylvaticus*, Blanice River floodplain.

T1.6 Wet Filipendula grasslands

NATURA: 6430 Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels

Unmanaged wet meadows often gradually turn into *Filipendula ulmaria* fallows (**Fig. 147**). The only stands which are important from the point of view of habitat protection are non-eutrophic stands, which are diversified in terms of number of species and have a sufficient representation of species of wet *Cirsium* meadows or, alternatively, wet *Molinia* meadows, from which they probably originated. Mowing is effective at greatly reducing the expansion of *Filipendula ulmaria*, and mown wet *Filipendula* grasslands very quickly turn back into wet *Cirsium* meadows. Wet *Filipendula* grasslands are not nutritionally important for FPM, unlike wet *Cirsium* meadows, from which they often develop and which are a suitable source of food for the mollusc.

The management of such stands is problematic. To achieve high-quality *Filipendula ulmaria* grasslands, it is ideal to leave them without regular management, but only if they are not affected by nutrient runoff and subsequent eutrophication. If the symptoms of eutrophication are very strong and there is an increased presence of ruderal species, for example *Urtica dioica* (stinging nettle), it makes little sense to start mowing or to commence other management unless the source of eutrophication is eliminated.

Otherwise, non-eutrophic stands in which *Filipendula ulmaria* predominates may be mown once every three to six years. More frequent mowing is not advisable because it suppresses *Filipendula ulmaria*. In any case, grazing has no place in tall stands with *Filipendula ulmaria*.

Managing *Filipendula ulmaria* meadows and activities associated with protecting FPM typically constitute a conflict of interest[2]. On sites (or in their parts) where the mussel is the object of protection, this should take precedence over management for the habitat of such fallows. In such places it is therefore appropriate to mow wet *Filipendula* grasslands and to transform them into wet *Cirsium* meadows (or other parental habitat types) that are more favourable for FPM.

T1.9 Intermittently wet Molinia meadows

NATURA: 6410 Molinia meadows on calcareous, peaty, or clayey-silt-laden soils (Molinion caeruleae)

Intermittently wet *Molinia* meadows (**Fig. 148**, **149**) are present in the Vltavský luh marshland and on the floodplains of the Blanice River and the streams Lužný potok and Zlatý potok. At present it is an exceedingly rare, sensitive, and highly endangered type of habitat. It reacts very quickly and sensitively to changes in the environment and the method of management. Over the past decade, the extent and quality of this type of habitat has decreased considerably as a result of environmental and management changes. The preservation of intermittently wet *Molinia* meadows in the current cultural landscapes is a major challenge in need of discussion. Such meadows are of great importance for FPM, especially those with a well-preserved structure and greater species diversity.

In the past, intermittently wet *Molinia* meadows were mown regularly, usually in late summer or early autumn. At present, experience seems to show that once mowing management is commenced,

[2] In SCIs where the simultaneous objects of protection are FPM and habitat type 6430, it will be necessary to resolve the conflict of interests in the SRM.

Fig. 146. Stands with *Carex brizoides*, Blanice River floodplain.

Fig. 147. Wet *Filipendula* grasslands with *Filipendula ulmaria* dominant, Blanice River floodplain.

Fig. 148. Intermittently wet *Molinia* meadows with *Succisa pratensis* (devil's-bit).

Fig. 149. Intermittently wet *Molinia* meadows – more nutritious types, near the village of Arnoštov.

it must not be interrupted and has to be repeated annually to avoid further degradation. However, regular annual mowing, especially if it is carried out at the same time for multiple years, does not suit indicator species of such meadows. It is necessary to design the management of meadows of these types experimentally and to approach each site separately. It is essential to evaluate each management activity annually and to draw up a plan for the next season. From the perspective of the protection of rare insects, it is often advisable to leave smaller parts of meadows, in the form of strips or islets, alternately unmown in the given year and mow them in the following year. Grazing on such habitats types is utterly inappropriate. Moreover, *Molinia* meadows react differently in different areas and it is not possible to unambiguously specify one type of management for all areas. It is necessary to set the management individually for each specific area, that is, for every occurrence of the T1.9 habitat type, and to adjust it every year depending on the situation.

9.4.6 T2 *Nardus* grasslands

T2.3B Submontane and montane Nardus grasslands without the occurrence of Juniperus communis (common juniper)

NATURA: 6230 Species-rich Nardus grasslands on siliceous substrates in mountain areas (and submountain areas in continental Europe)*

There are two different types of *Nardus* grassland vegetation in the Šumava Mountains, on the floodplains of the Blanice River and the Zlatý potok stream, as well as in the catchment of the Malše River. One type is represented by 'traditional' shortgrass *Nardus* grasslands, usually with predominant *Nardus stricta* (matgrass), whose stands in montane areas are sometimes very poor in species, whereas in submontane positions they are often richer.

The second type is represented by more mesic types of *Nardus* meadows (**Fig. 150**), where other grass species predominate, for example *Agrostis capillaris* (common bent), *Festuca rubra* (red fescue), or *Deschampsia cespitosa*. Other species present in such vegetation include species characterizing *Nardus* grasslands. Especially at higher elevations they are joined by indicative species of montane *Trisetum* meadows, for example *Bistorta officinalis* (common bistort), *Cardaminopsis halleri* (thale cress), *Centaurea pseudophrygia*, *Crepis mollis subsp. hieracioides* (northern hawk's beard), *Geranium sylvaticum*, *Phyteuma nigrum* (black rampion), *Cirsium heterophyllum* (melancholy thistle), *Silene vulgaris* (bladder campion), and *Viola tricolor* subsp. *polychroma* (wild pansy). All the species listed also occur in some other habitat types in Šumava Mountains. More mesic *Nardus* meadows sometimes represent transitional types of vegetation that are close to some types of montane *Trisetum* meadows in their species composition. *Nardus* grassland stands are of rather secondary importance for FPM in terms of food resources.

'Traditional' montane and submontane *Nardus* grasslands (**Fig. 151**, **152**) may exist for relatively long periods of time without management, although they may occasionally be grazed for a short time only.

A problem is posed by more mesic types of *Nardus* meadows. Grazing does not place a large eutrophication burden on such meadows, provided that they are grazed by a small number of cattle (0.5–1.2 LU per ha) during the day only. In such mesic habitats it is advisable to alternate different types of management (mowing one year, short-term grazing the next, etc.).

Fig. 150. Species-rich *Nardus* grasslands, Šumava Mountains.

Fig. 151. Submontane to montane *Nardus* grasslands, central Šumava Mountains.

9.4.7 L2 Alluvial forests

L2.1 Montane Alnus incana (grey alder) galleries

NATURA: 91E0 Mixed ash-alder alluvial forests of temperate and Boreal Europe (Alno-Padion, Alnion incanae, Salicion albae)*

Montane *Alnus incana* galleries rarely occur along the Vltava (**Fig. 153**) or in the upper stretches of the Malše (from its spring to the village of Cetviny). These communities include species of subalpine high forb vegetation. In terms of trophic level, montane alder galleries are of limited importance for FPM.

There is no need for any management in conventional non-degraded montane alder galleries. Exceptionally it is possible to perform selection cutting, preferentially of *Picea abies* and other naturally regenerating woody species. In degraded stands, where the spectrum of woody species does not correspond to a natural species composition, individual or group (targeted) selection of trees may be applied. However, it is always necessary to preferentially select spruce trees over alder, with the aim of achieving a natural species composition.

L2.2 Ash-alder alluvial forest

NATURA: 91E0 Mixed ash-alder alluvial forests of temperate and Boreal Europe (Alno-Padion, Alnion incanae, Salicion albae)*

This habitat type comprises mainly of alder galleries along streams with *Alnus glutinosa*, *Salix fragilis* (crack willow), and *Prunus padus*. Sometimes on sites of hillside springs there are galleries of association *Piceo-Alnetum* that harbour species also characteristic of wet *Cirsium* meadows. On the Blanice floodplain, there are transitional types of riverine and montane alder galleries with a marked presence of *Alnus incana* (**Fig. 154**). With regard to food resources, the most important are various types of spring alder galleries; those on the banks of larger water courses are of lesser importance.

If the water course is already shaded, selection cutting can be carried out. The same applies to woodland hillside springs and surrounding alder carrs with spruce. It is more appropriate to preferentially reduce *Picea abies* (and other naturally regenerating woody species) before *Alnus incana* and to prefer natural regeneration of all trees and shrubs of the original species composition.

9.4.8 Habitats strongly influenced or created by man

X5 Intensively managed meadows

Mown and grazed cultural meadows are found in all the catchments mentioned above. The importance of intensively managed meadows as a source of nutrition for FPM seems to be very small. The problem resides, among other things, in the transfer of detritus. Mown meadows need to be maintained by mowing and pastures can be utilized as they are. However, with regard to FPM, it is necessary to reduce the rate of fertilization to an acceptable level and to limit the amount of livestock

Fig. 152. Submontane *Nardus* grasslands, Blanice River catchment on the slopes along the course of Sněžný potok stream.

Fig. 153. Montane *Alnus incana* galleries on the banks of the Studená Vltava River.

to at least one-third of the original state within a distance of about 500 m from the river in order to avoid direct eutrophication in its vicinity. In some plots close to the water course (i.e., the buffer zone), it is best to entirely cease grazing and to provide management only by mowing. Compost and manure deposits are completely undesirable in the buffer zone. The same applies to larger tributaries of the actual FPM water course; however, the buffer zone around them may be half the size, depending on the overall character of the river and surrounding area. Ideally, more extensive management should be introduced in intensively utilized areas, leading to the restoration of species-rich meadow types. In addition, intensively maintained meadows and pastures often represent vast areas in which streams (mostly tributaries of FPM water courses) have been drastically regulated. In these cases, the optimal course of action is to restore the natural shallow, undulating channels of these tributaries by well-known and well-established means (Just 2003). From the point of view of the management of FPM, there is no need to take any other specific measures in these types of stands.

X7A Herbaceous ruderal vegetation outside human settlements, important conservation stands

This category of habitats mainly comprises unmanaged areas that are gradually becoming overgrown. Rarer species of plants still occur in only some of them. In terms of food resources, this type of vegetation is of no crucial importance for FPM. In relation to the pearl mussel, there is no need to deal with these areas in too much detail or to propose any special management. The mowing of these areas is important in eliminating agents of ruderalization and potentially aggressive species throughout the catchment. These species could eventually spread to natural habitats. One current problem in mesic habitats within the catchments is the rapid local spread of *Lupinus polyphyllus* (large-leaved lupine), an invasive species. Degraded stretches of meadows on which this species occurs with greater

Fig. 154. Alder stands along the banks of the Blanice River.

coverage must be mown repeatedly and biomass has to be removed diligently. The establishment of the species in still relatively well-preserved types of alluvial vegetation can be suppressed by its repeated destruction (weeding, mowing). How the occurrence of this invasive species is related to hydrologically affected habitats is not entirely clear, but it seems that, given the current dynamics of the spread of this species, measures to promote the quality of affected communities within the catchment are quite important.

X12A Stands of spontaneously established woody species

Over the past twenty years or so, nature conservation has also been addressing the problem of spontaneously established pioneer woody species in various types of natural habitats (**Fig. 155**). Woody species usually worsen the availability of food for FPM, so it makes sense to deal with spontaneously established vegetation in spring areas and small marshes, as well as with spontaneously established stands along water courses.

9.5 Meadow management in catchments with FPM

The methodology of meadow management in areas with FPM is a highly complex topic that must be addressed by a team of experienced specialists – not only botanists and ecologists, but also zoologists and experts from other professions.

There are a number of studies dealing with the ecological aspects of meadow vegetation management and its relationship to changes in species composition (e.g., Blažková 1989, 2003, Kučera 1995, Prach & Straškrabová 1996, Pavlů et al. 2003, Prach 2007). However, studies currently dealing with the relationship between vegetation management and the biology of FPM are scant. The only author that has addressed this topic is Blažková (Blažková & Hruška 1999, Blažková 2010). She has dealt with

Fig. 155. Naturally regenerated pioneer trees. In recent years, they have begun to be overgrown with *Galium aparine* (cleavers).

the situation on the Blanice River in great detail and has come to a number of important conclusions, which are still valid. However, vegetation succession at the landscape level has progressed since her experiments: at the time of her studies, the main species expanding into unmanaged meadows were *Carex brizoides* and *Filipendula ulmaria*. She pointed out that these species severely reduce the dietary supply for the pearl mussel and that their expansion is therefore a serious problem for maintaining its populations. Recently, the spread of these species has been accompanied by the extremely fast expansion of *Phalaris arundinacea*, and, in the catchments of the Vltava River and the Zlatý potok stream, also of *Carex buekii*. Also spreading in the Vltava River catchment is *Spiraea salicifolia*. All these species pose a further threat of reducing the dietary possibilities of the pearl mussel.

Drawing up suitable management plans for individual habitat types and catchments necessitates the initiation of an array of experiments, whose results must be monitored and evaluated. At present, expert monitoring of FPM populations is in some form ongoing in the areas concerned, but changes in vegetation (including the spread of expansive species that reduce its food supply) are not permanently monitored or the results of the monitoring are not available. If further management of meadow stands is commenced in areas with the occurrence the mussel, it is necessary to start monitoring vegetation changes and to evaluate them regularly. Only in this way can optimal management be established.

9.6 Targeted composting in functional plots within rearing and reproduction features

The management of functional plots established within the framework of breeding and rearing features on the streams Zlatý potok, Spálenecký potok, and Lužní potok, including the side arm of the Blanice River, currently includes a number of activities that are necessary for maintaining these features as refuges for FPM. One such activity is the establishment of special compost piles. The establishment of compost piles and the use of composted soil, which is taken back to plots within breeding and rearing features, is not integrated with the 'Methodology of meadow management' for the following reasons: it is immediately apparent from phytosociological relevés obtained at breeding and rearing features that adding composted soil increases the proportion of high-yield grasses (e.g., *Poa pratensis* – blue grass*, P. trivialis*, *Alopecurus pratensis*, *Holcus lanatus*). From the perspective of meadow habitat quality it is not desirable to increase the proportion of these grasses. However, from the point of view of pearl mussel nutrition, increasing the proportion of these grasses *is* desirable. It is therefore immediately clear that returning composted soil to certain types of habitat is not always appropriate from the standpoint of habitat quality[3]. Examining the effects of added compost soil on individual types of habitat will require a longer-term study.

Layers of cut turf as bases for compost piles are placed in shady and dry positions at least 10 m from the of the water course and about 250 m from each other. Turf bases for composting must be established with care, using the procedure below.

Composting is a good alternative of utilizing mown biomass, despite the increased human effort and cost. However, a certain disadvantage of special compost piles is slight ruderalization of the surroundings, which can be eliminated by mowing the surroundings of compost piles two to three times a year. Despite these slight drawbacks, this 'targeted composting' can be recommended as an alternative to storing meadow biomass at important sites.

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[3] Similar to *Filipendula ulmaria* fallows, the requirement to increase the nutritional value of grassland vegetation in breeding and rearing features may be in conflict with the corresponding SRM.

9.6.1 Establishment of a turf base

The turf base serves as an insulating and absorbing layer for composting mown grass biomass (**Fig. 156**). Its base (recommended dimensions 3.5 × 6 m) consists of turf placed grass side downwards, which is then compacted by raking and liming. Subsequent turf layers are composed in the same way (turf pieces in the top layer must be layered over the bottom ones to cover the gaps). In the last two layers it is necessary to reduce their overall size by one piece of turf on each side (the fourth layer is ca. 2.5 × 5 m). The consumption of ground limestone per turf base is 80 kg (24 kg, 21 kg, 19 kg, and 16 kg for individual layers from below).

9.6.2 Composting

Mown biomass is laid on to the composting bed in layers about 25 cm thick, first around the perimeter of the bed (wreath) and then in its middle. Using a pitchfork, mown biomass then need to be dragged from the middle to rim of the composting bed and folded over the edge to make it smooth. This serves to prevent over drying and leaching from the edge of the composting bed. A proportion of the determined total amount of ground limestone is applied to the surface and then incorporated. Used limestone must be ground finely without any lumps. It must be sprinkled evenly over the whole surface. Tapping a pitchfork to the layer of limestone dust makes it to stir up and then sink down into the biomass layer. When the biomass is correctly treated with limestone, it creates an alkaline environment, where ammonia ions contained in leachate is transformed to molecular ammonia that escapes into the air.

The process is repeated about ten times. The initial height of the first base is 2.5 m. After several days of settling, the final height of the first base does not exceed 150 cm. The maximum width of the composting bed must not exceed 2.5 m. This is very important for correct aeration of the biomass.

The finished base is covered with a tarpaulin (top and two sides are covered by overhanging cloth, two sides are open to allow access for air, **Fig. 157**), which provides shading and initiates composting processes. It also prevents fresh compost from being drenched by torrential rain followed by loss of leachate containing ammonia ions. During the initial phase of compost maturation (until the first re-sorting), the pile leaches liquids with a high nitrogen content. With composting beds made on a layer of turf, this leachate is absorbed by the soil of the turf base. In other cases, it is necessary to install collection containers that can catch the leachate. This can then be applied back to the composted mass during the first year of maturation. In subsequent years, it is possible to partly apply the leachate to the surface of an older compost pile. **Figure 158** depicts another variant of composting (with a roof); this is done at the Lužní potok stream breeding and rearing feature.

After the second mowing, tarpaulin and the over dried top layer of the first base is removed. Ovedried biomass is mixed with new fresh biomass. The second base is created by the same method as the first base. This cause significant raise of the height of the base surface, subsequently the appropriate ladder is needed for proper application and incorporation of the limestone.

It is important to notice that this process does not create just any compost for the garden, but a special compost for the FPM biotope, which can be beneficial or also very dangerous. Therefore, it must be very carefully ensured that all work is carried out in the right quality and with great caution against soil and water contamination. The surroundings of the compost beds need to be continuously checked by conductometry. Improperly made composts must be used on areas that are not in direct contact with RRF waterways.

Fig. 156. Compost bed made incorrectly from pieces of turf loosely placed on a tarpaulin. Correctly made compost does not need to be placed on a tarpaulin sheet to stop leaching of ammonia. When the biomass is correctly treated with limestone, it creates an alkaline environment, where ammonia ions contained in leachate is transformed to molecular ammonia that escapes into the air.

Fig. 157. Establishing a composting, Blanice River – SRRF. The plastic pipe with holes serves to facilitate the aeration of the composted material. It is placed along the centre of the compost, at the top of the composting bed.

9.6.3 Compost overlayering

The overlayering of compost is done each year in autumn (from early November until the first frosts) and in spring (after the thawing of the base, by the end of April at the latest, to allow reptiles to hatch undisturbed in the base). Before the overlaying, the previous layer is removed from the compost to the nearby tarpaulin sheet, where compost is checked for undesirable limestone lumps, and dry topmost layers of biomass are mixed with inner layers (and wetted if necessary). This biomass is then reaplied on the one half of the compost bed area. After the second overlayering, the compost mass no longer releases hazardous leachates, so it is not necessary to use a protective tarpaulin.

After three years of being piled, the compost is mature (**Fig. 159**) and can be re-applied to functional plots.

9.6.4 Re-application of compost to functional plots

Mature compost is used to improve the soil conditions of functional plots. This is done after the quality control, because an improperly made composts can be dangerous to the FPM biotope and must not in come into direct contact with RRF. Compost is then re-applied to the land in the spring of the fourth year after its piling, together with the fifth overlaying. The compost mass is placed along the banks of the water course in 1 m wide strips (**Fig. 160**).

The end line of the functional plot with composted material must be marked in the field in order to maintain continuity of application in the following year.

In the sixth year after the start of composting, the turf base is replaced with a new one. Turf from the original base is spread over a suitable plot of land in a layer and area determined by evaluating the state of vegetation and the exchangeable soil acidity of this plot. Finally, after an eight-year meadow management cycle, the compost bases are removed.

Fig. 158. Composter with a roof, Lužní potok stream.

Fig. 159. Composting at Spálenec SRRF. One-year-old compost at the front, behind (in sequence) two and three years old compost.

Fig. 160. Re-application of compost along a stream.

9.6.5 Use of composting in the management of small protected areas

The establishment of targeted compost piles might prove useful when caring for small-scale protected areas, especially where access for machinery is limited, making it difficult to reliably remove mown biomass. It is a natural alternative that could help to save some valuable areas for which no conservation management has been proposed because of difficulties concerning machinery access. When establishing targeted compost piles in the vicinity of small-scale protected areas, it is always necessary to have the whole situation assessed by an expert – a botanist. It is possible that the re-application of composted soil is the right type of restoration management in certain special cases, for example in the reconstruction of stands with *Carex brizoides*. However, this issue requires special monitoring and evaluation with respect to both FPM and the natural habitat.

Chapter 10: Special restoration, renewal of the water regime, and special measures

Authors: Mgr. Kamila Tichá Ph.D., Mgr. Ondřej Simon, Jaroslav Hruška, Mgr. Jan Švanyga
Reviewers: Ing. Dagmar Brejšová, Ing. Tomáš Just

This methodology for restoration of river catchments with the last occurrence of freshwater pearl mussel has been prepared for landowners and operators, administrators of watercourses, and authorities for both nature conservation and protection of waters. Its purpose is a sensitive management of the environment of a globally threatened species and, therefore, the methods used are often different from normal restoration.

The method assumes that **management of small watercourses and their water regime is to be implemented at the lowest possible level**. If the local population, land owners, or regional conservationists have detailed information available to them, they can more effectively protect the purity of water and entire water ecosystems (including critically endangered freshwater pearl mussel) than officials and authorities from remote locations.

In some river catchments, this has been successful in the long term, and it is possible to see a number of cases of smaller measures and restorations, which were carried out, so to say, from below. Many others were carried out on the basis of fulfilling management plans for protected areas or in close cooperation with watercourse administrators and nature conservation. The first major restoration projects have also been successfully implemented. The precise procedures of the individual measures need to be planned or, where appropriate, designed specifically for the given river catchment and location. This methodology provides an overview of measures that can be used.

It is often difficult to understand extensive management plans and their annexes for large protected areas. This text provides a brief overview of simple and, in many cases, inexpensive measures which have proven their worth and can be easily applied in river catchments with freshwater pearl mussel (**Fig. 161**).

Fig. 161. A mosaic-like catchment with a multifarious mixture of wetlands, spinneys, and meadows. FPM can only survive in such varied catchment areas with a mixture of forest and non-forest habitats permeated with clean and natural streams.

As part of the preparation, whether classic restoration of medium and large streams or minor restoration interventions of small streams, it is appropriate to realize that these actions always require detailed pre-project preparation and a project prepared by a designer with experience of sensitive upland conditions. All restoration projects in Czech Republic are subject to a water-management building permit and in protected areas are carried out under the supervision of the Nature Conservation Agency. Large interventions (technical restoration) in watercourses restoration with FPM should be incorporated into the River Basin District Plans well in advance. In addition, multiannual biological research and other necessary evaluations need to be carried out in a timely manner. This process is long but necessary.

Large structures (worth tens of millions CZK) will only be mentioned marginally in the following text (this is always a site-specific project). We focused more of our attention on small measures, such as the seepage of a road ditch or the local reinforcement of an excessively eroded bank with live willow fascine bundles; these are inexpensive, simple measures and need to be implemented in large quantities.

The text is limited in length and therefore cannot describe the issue in its entirety. For a comprehensive approach to a topic that goes beyond a narrowly focused view of one species, we recommend the relevant literature (e.g., Just 2005).

10.1 Restoration measures for freshwater pearl mussel

In this chapter, we will focus on restoration measures for watercourses, spring source rills, ditches, and concentrated water outflows, and will show which deficiencies can be corrected by restoration or which characteristics of the river network can be improved. Several major causes of FPM disappearance have been identified across Europe (Geist 2010). In addition to water pollution, it is primarily erosion (**Fig. 162**) and the consequence of excessive flushing of clay and sand particles into rivers, in particular from ditches, eroding gullies, and deepened streams, from roads, tracks on slopes, and forest clear cutting (**Fig. 163** and **164**), as well as urban areas (Österling et al. 2008, 2010). For many pearl mussel locations, revitalization measures can often be carried out simply, and erosion practically does not have to occur.

As a result of the release of fine particulate clay into the catchment, clogging occurs of the interstitial spaces on the stream bed; the quantity of oxygen decreases here, putrefaction begins (releasing toxic substances), and juvenile and sub-adult FPM cannot survive (Geist & Auerswald 2007). The population disappears step by step due to such erosion. Clogging of the stream bed is a gradual phenomenon that is not evident at first sight but can be documented by a detailed measurement over many years.

In contrast, sand clearly damages sites where adult FPM live, as shown in **Figures 165** and **166**. Stable stream bed with adults can exist for dozens of years under normal circumstances in protected meanders. If the colony is overwhelmed with sand, the pearl mussels must leave their previously stable site and climb to the surface of the sand. However, they can withstand neither storm events nor springtime floods, and are flushed into the unfavourable depths or washed ashore, thus destroying the colony.

These cases were recorded at the end of the 1980s on Tetřívčí potok stream, on Malše River after 2002, and on the Blanice River below Zbytinský potok stream in 2004–2006 (Spisar & Simon 2006). Currently, sand erosion from multiple sources significantly threatens the population in Zlatý potok

Fig. 162. Erosion and its consequences. In unstable subsoil, artificially deepened channels with a depth and width of 0.5 m erode quickly into a gully up to 2 m deep (Vyšenský potok stream 2005, water level at normal flow rate). Coarse stones, if present, remains in place.

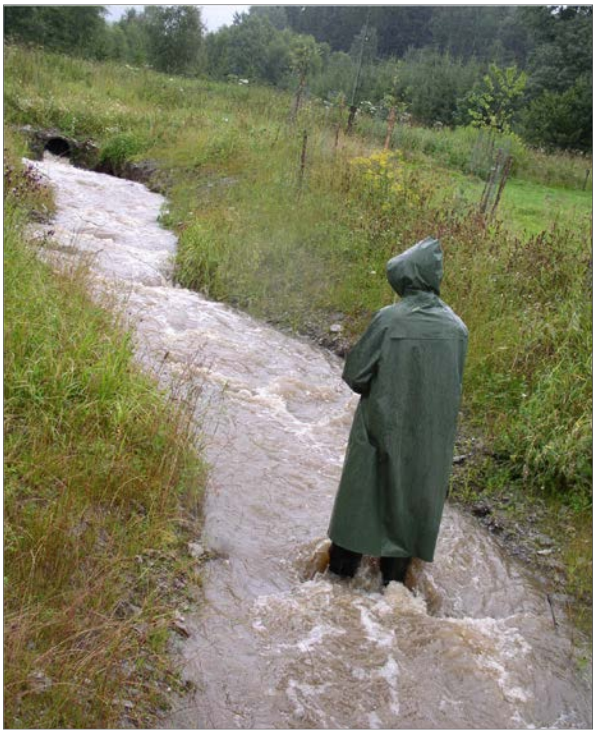

Fig. 163. Ablation of sediments. Flooding can transport fine clay sediments over a long distance into larger streams, where they clog the porous stream bed.

Fig. 164. Example of the origin of erosion. Large-scale land management can be insensitive to small watercourses and deadly for FPM. Forest harvesting performed regardless of waterlogged ground can cause pollutant run-off into watercourses and massive erosion.

Fig. 165. Deterioration of the riverbed by sand deposits. Massive erosion from Kabelský potok stream and other sources in the catchment completely clogged the originally solid gravel bed of the Malše River.

Fig. 166. The border part of the Malše River, where ideal sediments have been maintained with the remnants of FPM population in the last unpolluted section of the river (an extinct settlement of Horní Příbrání, 2015).

stream and the Malše River. Remedying this unfavourable situation and removing the deposited sediments from the river is very difficult, and will take a long-term, systematic effort (Altmüller & Dettmer 2006).

10.1.1 Structural restoration of watercourses

Modifications of watercourses, their regulation, and ameliorations (mainly in the second half of the 20th century) also has a significantl effect on the condition of streams with FPM. Some less drastic regulative measures were implemented in earlier times, for example in connection with timber floatation. In this way, relatively long sections of the Vltava and Blanice Rivers were fortified with rockfill. At present, the collapsed remnants of stone walls no longer have a stabilisation function and can have a favourable ecological impact, for example as a shelter for fish or even as a suitable habitat for FPM, which find a stable and secure footing among the rocks.

Later modifications of watercourses were more drastic. The channel was usually completely straightened and deepened to accomodate inflows from drainage systems. In river catchments with FPM, these are currently mainly tributaries because the freshwater pearl mussel is no longer present in the heavily regulated areas due to their habitat requirements. The exception is part of Lužní potok stream and the Bystřina stream (both in Aš region), which are straightened and deepened in the upper course, i.e. above the pearl mussel sites. On Lužní potok stream, restoration is not yet completed, and the restoration of the Bystřina stream is in its feasibility study phase (Beleco 2016). As far as the other structural works are concerned, total restoration of a one-kilometre upper section of Luční potok stream is currently planned (tributary of FPM locality Zlatý potok stream), for which a feasibility study was done in 2016 (Beleco 2016).

In this methodology, the concept of structural restoration refers to the conversion of a technically altered channel into a semi-natural state, while the very concept of restoration here has a broader meaning because it also includes minor modifications, for example special restoration of the spring areas and restoration of fine hydrological networks of of catchments.

In the case of FPM streams, even the question of the spontaneous restoration of the flow, or possibly semi-natural restoration, where man simply starts natural processes and the river itself then creates its new channel, is questionable. These variants can be several times cheaper than structural work, which creates a completely new channel. Spontaneous restoration of a functioning watercourse, which can occur where there has been relatively little disruption, can be of course considered a positive phenomenon. If the disruption was drastic (the channel was straightened, deepened, and fortified), spontaneous restoration of a stream cannot be assumed and **the option of human assisted restoration (e.g., removing concrete lining from the stream bed and banks) is not a suitable solution in the case of FPM streams**. Simple removal of concrete walls may increase erosion in the short to medium term so that even an otherwise relatively insignificant tributary may put pearl mussels at risk. This case is described in detail in **Chapter 10.1.3.1**.

10.1.2 Structural restoration of streams, its significance, and explanation of when it is required

The implementation of structural restorations is conditional on the proper project development and is subject to all legal processes like other constructions based on a water management building permit. In the case of FPM streams or their tributaries, the extraordinary sensitivity of the FPM to environmental damage must be considered when drawing up the project documentation and its assessment by the nature conservation authority. It is known that, in the short term, an adult individual FPM can survive both the drying up of a stream and increased sediment or chemical loading due to its ability to close its shell, unless the pollutants are strictly toxic (Degerman et al. 2009). However, this does not apply to juveniles and, as described in the introduction, juveniles are particularly sensitive to the oxygen content of the porous stream bed. Therefore, if more fine sediments are washed away during or after restoration, the pearl mussel would be in immediate danger. During restoration, it is therefore appropriate to build a new 'dry' channel and release water into it very slowly and gradually, under constant supervision.

It is often possible to trace the path of the original, pre-regulation channel in the landscape and then use this channel for restoration. This is useful not only in terms of preserving the "landscape memory", but also in other respects. The original channel usually leads through the most suitable thalweg and, after removal of the introduced soil, it can be relatively well stabilized, so there is no undesirable scouring and removal of sediments. Nevertheless, even with the most careful methods, the removal of sediments cannot be completely prevented, especially during increased flow rates – it can only be minimized. Each new channel is further developed by spontaneous processes and stabilizes for several years. Therefore, it is necessary to think in advance of possible measures that will help to protect the FPM population (see measures in **Chapter 10.3** – sedimentation traps, spills into vegetation, semi-permeable dams, pools, etc.). In an extreme case, the rescue transfer of the endangered population to another place may be undertaken, if the nature protection authority deems it necessary. However, every transfer involves a great deal of stress for the pearl mussels, so it is necessary to assess all circumstances responsibly.

10.1.3 Examples of restoration

10.1.3.1 Zbytinský potok stream and Sviňovická strouha stream in the Blanice River catchment

In 2004–2005, approximately 1 600 m of Zbytinský potok stream (tributary of the FPM locality Blanice River) and its tributary Sviňovická strouha stream were restored (**Fig. 167**). The project did not respect the requirements for restoration modification in a way that would prevent excessive transport of sediments to the Blanice River during the work and subsequent completion of the restored channel. In contrast to the initial requirement to create a shallow, undulating channel, all the reinforcement features in the deepened channel were removed, resulting in a non-stabilized, deep channel which was left to spontaneous erosion processes (AOPK ČR 2007, **Fig. 168**). The consequences for the FPM population and remedial measures in the form of a combined facilities for capturing sediments are described in **Chapter 10.3**.

10.1.3.2 Hučina stream, Žlebský potok stream, and Jedlový potok stream in the Vltava River catchment

The restoration of the Hučina stream (tributary of Studená Vltava River) was done in 2013 (**Fig. 169**)**,** with restoration of Žlebský potok stream (right tributary Teplá Vltava River) and Jedlový potok stream (left tributary of Teplá Vltava River) in 2014 and 2015. For the entire area of Vltava floodplain between

Fig. 167. Zbytinský potok stream and Sviňovická strouha stream (confluence) immediately after restoration. On the left side, next to the channel of the Zbytinský potok stream, the remains of the concrete bank protection of Sviňovická strouha stream are visible on piles; the Sviňovická strouha stream remained deep and straight.

Fig. 168. Massive erosion processes started by increased flow rates during springtime snowmelt on the restored Zbytinský potok stream, causing the erosion and transportation of fine grain material, sand, and gravel into the main flow of the Blanice River.

the villages of Lenora and Nová Pec, a comprehensive quantitative study was prepared in which an analysis was made of map data and, in a second step, an analysis was performed focused on the need for restoration of given sections or micro catchments. The result of the first analysis was a summarization of the length of the modified streams according to the flow order in four categories: (a) piped, (b) paved (stream bed, or stream bed and banks), (c) only route change or deepening, and (d) natural or slightly modified flows.

Restorations are also being proposed for other streams in the area below the confluence of the rivers Teplá and Studená Vltava (Starý potok stream and Chlumský potok stream in parts from the road to the junction with the Vltava River); they are currently in the phase of underlying studies. Restoration of Volarský potok stream is also being considered; older land use planning documents already exist, but they are not suitable with regard to the significance of this source of risk (Hladík et al. 2015).

Even before the restoration, the Jedlový potok stream and the Žlebský potok stream already had a chemistry similar to the main stream. Jedlový potok stream carries old agricultural burdens from the river catchment while, before restoration Žlebský potok stream in times of torrential rain and floods carried twice the concentration of sediments than the main stream. Restoration of all three tributaries of the Teplá and Studená Vltava rivers was treated very carefully, and sedimentation pools were built in several places to prevent sediments from entering the main river. Where possible, the new channel followed the historical, pre-ameliorative channel. Elsewhere, a completely new meandering channel was created. In several places, the channel was not excavated by the machinery to full depth and width, and the stream modelling was left to spontaneous processes (**Fig. 170**). Given that

Fig. 169. The newly restored Hučina stream with a carefully selected meander route, where it intersects the Povltavská stezka trail and Černý Kříž – Kájov railway line. The Studená Vltava River is visible on the right side of the picture. Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

the streams are located in the flat floodplain of the Vltava River alluvial woodlands, their gradient is very gradual and no significant erosion can be expected, even during increased rainfall or springtime thaw, when the Vltava River alluvial woodlands are completely flooded. Planting of accompanying tree species along the banks was not implemented for the same reason – herbaceous vegetation dominates the meadows, with trees appearing mostly as a riparian vegetation along the main stretch of the Vltava River.

10.1.3.3 Lužní potok stream

In 2016, the alluvial woodland section of FPM locality Lužní potok stream was restored, from the forest to the bridge below the village of Pastviny. The channel was deepened, straightened, and fortified here. As part of the restoration project, a new separate channel with a 'natural' meandering route is being built on the left bank next to the original straightened Lužní potok stream channel. The straightened channel is kept for emergency use after the springtime thaw or torrential rains. The shape of the channel provides good feeding opportunities for FPM (shallow, well stabilized stream bed, many meanders); the whole channel must be stabilized so that no large-scale erosion and sediment runoff take place, even in the early years (Řepa 2010).

Fig. 170. The steep mouth of Žlebský potok stream into the Teplá Vltava River. The channel has been modelled and reinforced with stones, unlike the flat floodplain where there was room for the water to spontaneously form the channel.

10.1.4 Parameters monitored and evaluated in connection with structural restorations

Structural restorations are extensive interventions in watercourses; therefore, it is necessary to physically and chemically monitor the parameters of the water before, during, and after implementation. As in the case of strengthening meanders and other major works, it is advisable to supplement this measurement with bioindications (for more information on bioindication method, see **Chapter 7**) and regular inspections of the FPM population (if it is located immediately below the implementation site).

When the measures are completed, it is advisable to use regular walkdowns to check the status of the restored stream and the amount of sediment carried by the stream, as well as after heavy rain and floods. As shown by the restoration examples, in the design phase it is necessary to think about possible erosion of newly built channels and to consider possible measures in advance that will minimise the impact on FPM. At the same time, it is necessary to inspect all restoration features that are implemented in connection with minimizing adverse impacts on FPM (sedimentation pools, spills, side arms, etc.). It is advisable to monitor the dynamics of the restored channel for a few years after restoration.

10.2 Rearing and reproduction features (RRFs)

10.2.1 RRFs, their significance, and implementation

Rearing and reproduction features (RRF) are secondary side arms of watercourses for young FPM built on gravel substrate (**Fig. 171**). Their importance stems from the need to create an appropriate and easily controllable environment with good substrate quality and food quality, especially for freshwater pearl mussel juvenile stages. For more about the operation of food components, see **Chapter 5**.

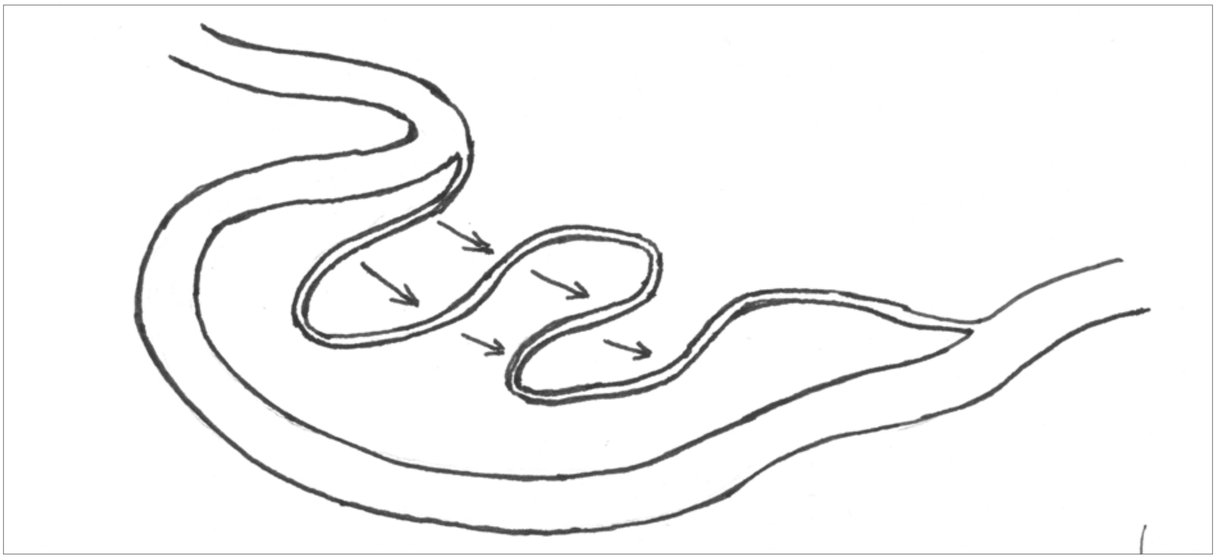

Fig. 171. RRF scheme – a meandering channel crossing a meadow and its connection with the main stream. The arrows indicate the direction of groundwater flow through the rhizosphere (rooted zone of meadow vegetation on porous gravel) across the meanders. Illustration: Michal Bílý.

Building a RRF must be preceded by the creation of detailed project documentation. The location of an RRF is selected based on slope, soil fertility, insolation, and tributaries – potential food rills. If there are sufficient natural fluvial deposits in the RRF location, the RRF can be built without an artificial gravel bed. If the construction of an artificial gravel bed is necessary, the soil is removed from the entire area to a depth of approximately 40 cm and a bed is made of coarse gravel with a thickness of approximately 20 cm; the channel is then lined with wooden boarding (**Fig. 172**). The boarding is removed after filling with carefully treated cover soil (with the correct pH and Ca content), sowing and good rooting of the banks. Sowing must be carried out with a mixture of seeds of local origin so that the resulting meadow habitat meets the demands of the FPM for the composition of detritus food chain (see **Chapter 9**). The controlled flow rate, together with growth of the bank vegetation (both sides), will lead to gradual optimal channel formation (**Fig. 173**). The construction takes 4–5 years and must be carried out with regard to natural processes (Dort & Hruška 2008).

After several years of natural influences, such as natural completion of the RRF channel and spontaneous growth of corresponding flora and fauna, the auxiliary lateral branches of streams can allow optimal natural reproduction of FPM and their subsequent spread into the main stream. In most cases, when the state of the catchment is unfavourable for reproduction, it serves for a long-term site for planted FPM (several years old) from a semi-natural RRF; they live here under the stream bed until they spontaneously leave the river branch between the ages of 10 and 30 (when they are already living on the surface and need a greater flow).

The role of RRFs as refugia (refuge from unfavourable conditions) and reserve gene pools in unexpected events (e.g., accidents, floods) in the main stream is not negligible. Thus, a well-functioning larger RRF can simultaneously fulfil all the reproductive functions of the pearl mussel, including a refugium and a reproductive environment for host fish.

10.2.2 Summary of completed RRFs

On the basis of the above described needs, four RRF have been built within the implementation of Action Plan measures: in the catchments of Lužní potok stream (LRRF), Zlatý potok stream (ZRRF), Spálenecký potok stream (SRRF), and the Blanice River. A somewhat different system was founded in

Fig. 172. The construction of rearing and reproduction features. SRRF – Blanice NNR – a layer of enriched soil with turf is visible along the future route.

Fig. 173. LRRF – Lužní potok NNR – around the still dry channel, gradually rooting turf can be seen. The remaining areas of both RRFs (Fig. 172) will then be covered with pre-composted soil enriched with calcium in organic form (See Annex 4 for possible errors in construction).

the original branch of the Blanice River at the village of Spálenec, called after the cottage, where part of the RRF had already been done, as Spálenec RRF centre (AOPK ČR 2013a, **Fig. 174–177**). Detailed comments on the function of the individual RRF for 20 years after their construction are given in **Annex 4**.

Blanice River – RRF Spálenec (= RRF centre). In the 1990s, a smaller pilot feature was built with an artificial gravel surface from the pool to the branch, fed partially by water from the Blanice River and partially from a small tributary. Practices improving the input of detritus to the flow through rills on the gravel bed were first tested here. In 2015, it was restored without connection to the small tributary and operates only on water from the Blanice River. The entire system now consists of a small side channel on artificial subsoil, a large branch of the Blanice River, and a complex system of so-called

Fig. 174. Functioning RRF. The smallest SRRF in the Blanice River floodplain. Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

Fig. 175. LRRF on Lužní potok stream (the dashed line marks the Czech Republic-Germany border). Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

Fig. 176. ZRRF on Zlatý potok stream – here without feeding rills on Zachráněný potok stream (right hand tributary of Zlatý potok stream in the village Miletínky). A side pool can be seen on the far left at the inlet to the branch of the Zlatý potok stream (ZRRF). Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

rearing rills. Thanks to its location on the porous sediments of the Blanice River, groundwater flow between the main stream and the branch is important here.

LRRF. In 2000, construction of the first of the special rearing and reproduction features on Lužní potok stream in cadastral territory of the village of Pastviny was completed (**Fig. 175**). Within two years, a meandering channel and adjoining food rills were built in an artificial gravel bed with enriched soil cover, with the aim of improving food supply in Lužní potok stream. In addition, Pod pásem, Nad pásem, and Za Pastvinami food rills have been built.

ZRRF. In 2001, an RRF was built on Zlatý potok stream in the cadastral territory of the village of Křížovice u Ktiše (**Fig. 176**). For the purposes of the Action Plan a 630 m side branch was created in natural gravel sediments of the floodplain, to which juvenile FPM from rescue rearing were relocated

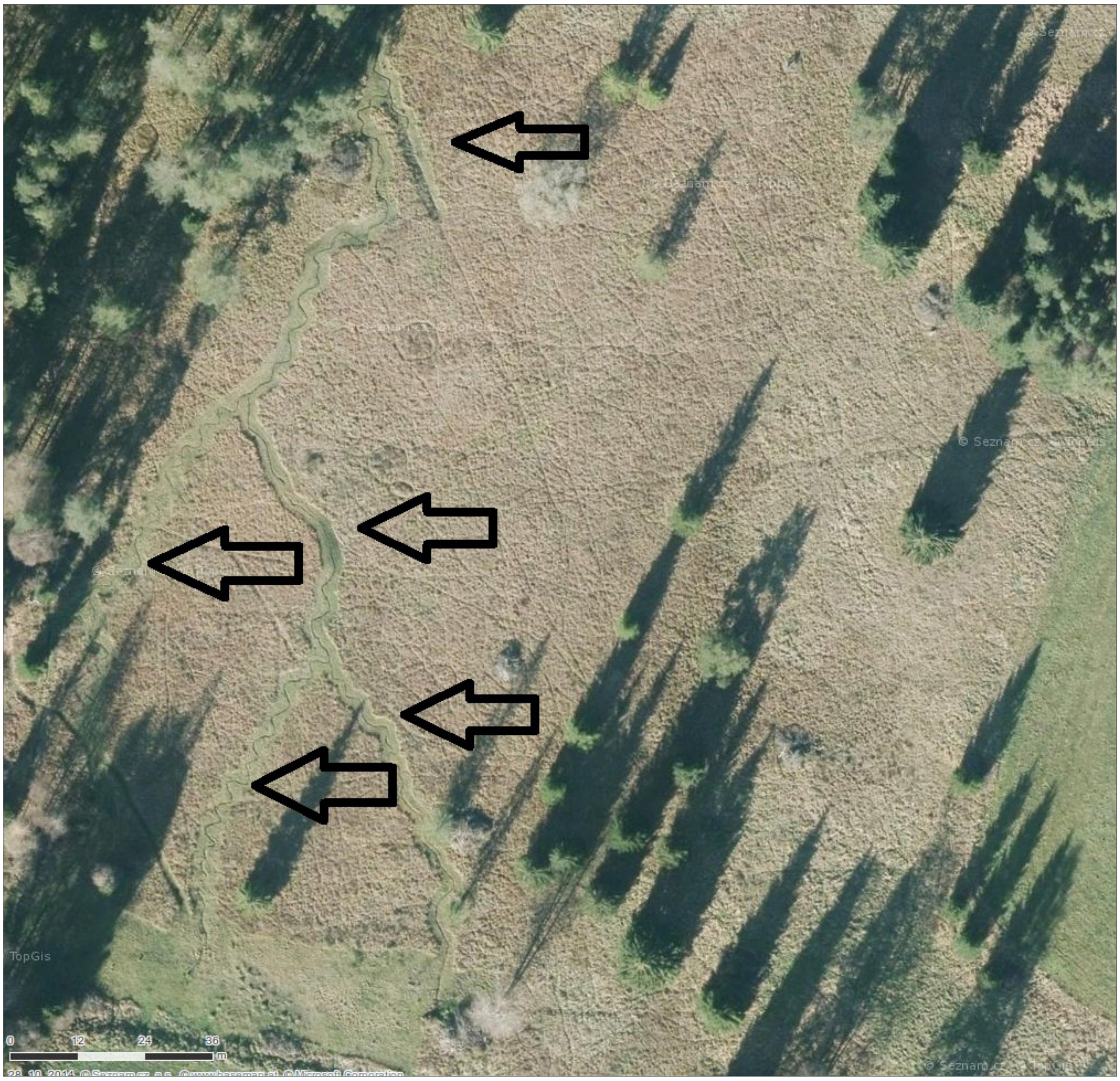

Fig. 177. For comparison – part of the rearing rills (marked by arrows) in the Sněžný potok stream catchment, left side tributary of the Blanice River below the village of Spálenec (Sněžný potok stream is hidden in the top left under the trees). The scale is identical to Figures 174–176. Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

in 2002 and 2003. In 2003, additional wetlands and food rills were built on sloping meadow land to improve detritus supply to the RRF.

SRRF. In the 1980s, the inflow of Spálenecký potok stream into the Blanice River below the village of Arnoštov was shortened and, thus, part of the floodplain with suitable soil and vegetation properties was closed off, which represented a very high-quality reproductive environment for FPM (**Fig. 174**). This function was partially restored by building an RRF in an artificial gravel bed in 2004. This feature is the only one that does not contain supplementary food rills. In 2005, juvenile FPM from rescue rearing were placed in the RRF.

10.2.3 Operational maintenance of RRF and monitored parameters

A number of parameters are monitored annually at individual RRFs (see **Chapter 5** for details).

Among other things, it is important to check the function of food rills and other auxiliary features throughout the year, especially **flow rate** of the branch. If necessary, flow rate of the rills can be controlled by means of wooden gates and sluices. After higher flow rates, it is advisable to carry out additional checks and, if necessary, remove sediment and fluvial deposits. At low flow rates, it is advisable to check the correct location of the reared FPM. At low temperatures in winter, it is necessary to check the condition of the sites, in particular due to the **risk of formation of anchor ice** (ensuring the branch inflow does not freeze) and **ice floe** (prevention of ice barrier formation). In times of intense ice phenomena, a daily check of the lateral branches is required prior to the formation of ice cover. Features should be inspected regularly at times of flow fluctuations. At several-years' intervals, it is necessary to check or adjust the integrity of food rills against seepage or make minor adjustments to the banks and maintain their optimum width. If food rills become overgrown, it will be necessary to cut back the unwanted vegetation.

In terms of the quality of the aquatic environment, it is advisable to carry out year-round monitoring of physical and chemical water parameters, in particular the basic measurements of water temperature and conductivity and, if necessary, to monitor water turbidity and nitrate load. If the relevant RRF is fitted with a telemetric station for recording continuous measurement of water level, water temperature, and conductivity, it is necessary to carry out regular checks of these stations in the field, to evaluate regularly collected data and, above all, keep mobile phones on standby to receive error messages. The operation of telemetric stations is not expensive (energy, web hosting), but labour intensive (Simon et al. 2010b, AOPK ČR 2013b, 2014). An RRF without a dividing feature has lower demands for daily management, such as SRRF, through which the Spálenecký potok stream flows in full volume.

10.2.4 RRF problems – current technical condition, repairs, and improvements

Management of individual RRFs is time-consuming and requires a constant presence of a responsible person on site or at least in the area. Failure of management can have fatal consequences, such as the destruction of the whole side-branch population, as has happened abroad (an arm of the River Our in Luxembourg). Therefore, it is necessary to approach the development of RRF with the knowledge of the necessary financial and organizational provision for management in the decades to come, and not forgetting that side branches are only an auxiliary temporary feature until the restoration of the entire river catchment.

10.2.4.1 Floods and RRF

Large floods can cause damage both to the RRF facility itself and to the FPM living there. If the RRF is set up correctly, there is usually only minor damage. In the case of the side branches, the effect of the main flow is apparent during floods, which prevents the inflow of water from the side branch because its channel is full including the overflow. This significantly reduces the risk of erosion in the side branch and flushing of the pearl mussels. Partial danger only arises after the flood has subsided, when the side branch drains into the main flow from the overflow in the flood plain. A smaller flood can also be controlled by a properly designed inlet feature.

Pearl mussels living in RRFs are particularly at risk from the amount of sediment transported by the current during floods, which is mainly a problem at RRF Spálenecký potok (not a classic side branch, it is built at the tributary). When larger objects move in the channel, there is a risk of physical damage to the shells. Due to the strong current, both juvenile and adult FPM can be carried downstream, where conditions may no longer be suitable for them. A good solution is to build a catchment place below the RRF, where the FPM cannot be washed away.

Major floods in recent years have caused little damage to hydraulically well-constructed RRFs. For example, at the beginning of June 2013, the Blanice River and Zlatý potok stream catchment endured a 'fifty-year flood' (**Fig. 178**). The water carried a large amount of sand, tree trunks, and wood. On the side branch of the RRF centre at Spálenec, a colony was washed away and the stream bed damaged – the subsoil was subsequently repaired, the deposits were removed, and the colony re-aggregated. The problems in this previously stable refuge, which withstood the major (‚one-hundred-year') flood of 2002, were probably caused by a heavy and firmly anchored footbridge low above the stream, and

Fig. 178. Examples of problematic incidents at an RRF. A 'fifty-year flood' on the Blanice River in 2013. Photo: Ondřej Spisar.

a wooden trough also firmly anchored into the banks that prevented the free flow of swollen water. Previously, there were only light, unanchored objects that were easily washed away by the swollen water, so there was no similar damage to the stream bed. In 2013, however, drifting branches and trunks became jammed and severe erosion of the stream bed occurred, including the destruction of the most valuable FPM colony. After the colony was re-established, it was no longer possible to create comparable habitat conditions. Therefore, paradoxically, a good solution in a flood plain are transverse obstacles above the channel that can easily be carried away by the current; they are always caught in the nearby vegetation and can be easily brought back. Compared to this site, the damage to SRRF in 2013 was only minor. At ZRRF, the bank broke off at the inlet feature. The inlet to ZRRF was completely closed off, but the channel of the feature was still flooded with a seepage from Zlatý potok stream (AOPK ČR 2014).

10.2.4.2 Drying out of RRFs

In catchments with a low amount of water which suffer from drying out in places, especially in the summer months, there is a problem with rapid backfilling of food rills by soil, which need to be renewed regularly. In the case of short term, insufficient flooding of food rills, the amount or quality of washed-out detritus may be reduced, which is unlikely to affect FPM due to the natural fluctuations in the quantity and quality of detritus during the year. If the main stream dries out, immediate action is necessary (e.g., rescue transfer). If the cause of drying is a disrupted water regime, it is appropriate in the long term to address the situation with complete restoration of the river catchment. If there are other causes (e.g., extreme weather fluctuations caused by climate change), it is necessary to consider, on the basis of relevant data, whether the habitat is still suitable for FPM.

In the Lužní potok stream catchment on the LRRF, for example, there is usually a lack of water right from the beginning of summer and food rills are filled with soil. Most of the rills have been modified in recent years, and the clogged bypass branch at LRRF has also been restored.

In the summer 2003, Bystřina stream channel dried up, so that it was necessary to carry out a rescue transfer of all affected pearl mussels to LRRF. When the situation improved, all the individuals were returned to their original habitat (AOPK ČR 2007). A similar transfer happened in 2015. It can thus be confirmed that RRF work reliably as refugia for FPM under adverse hydrological conditions.

10.2.4.3 Ice formation in RRFs

Another problem that can be caused by meteorological phenomena at RRF is the development of anchor ice in the side branches (**Fig. 179**). It is therefore necessary to perform regular field inspections during severe frosts and, if necessary, to break up any ice at the inlet feature. Very strong ice phenomena were recorded in the rill of RRF centre in winter 2001–2002, but timely intervention prevented FPM losses.

10.3 Trapping eroded sediment

Excessive inputs of sand and suspended fine clay particles into a stream are mainly caused by erosion after inappropriate alteration of small tributaries or construction of drainage ditches. This can have a critical impact on the survival of FPM, especially in juvenile stages. Coarser particles, especially sand, can also clog larger adults that are firmly seated in the gravel bed. In an effort to keep their filter apparatus in contact with running water, these individuals often leave their habitat and may subsequently be washed downstream to a less suitable locations. In addition to sand, fine clay deposits are also dangerous to juveniles; they clog interstitial spaces in the stream bed, and dramatically reduce the available oxygen in the hyporeal. On the Lutter River in Germany, it was discovered that excessive deposits of suspended solids and sediments from drainage ditches were among the causes of juvenile survival problems in the main stream (ploughing can result in more than 1 m^3 of sand per week being brought into the main course). As a result, sand traps and treatment ditches with wetland plants were constructed on all drainage ditches leading into the river and its tributaries. The erosion of arable land is also undesirable for farmers; therefore, the prevention of introduction of sediments is often in the interest of those managing agricultural land around streams. Natural features that can be seen on natural floodplains are often a model for erosion trapping measures (**Fig. 180**, **181**).

These minor measures can be provided in a larger catchment area by a contractor, provided that there is no risk of adversely affecting the protected habitat. If this is a possibility, it is necessary to monitor the flow before, during, and after intervention using automatic continuous measurement associated with reporting of critical values exceeding selected limit indicators. It is always necessary to provide expert supervision by a legal or natural person with the knowledge and documented practical experience in the protection of oligotrophic catchments with FPM (Simon et al. 2010b).

10.3.1 Sedimentation pools

Suspended matter and sediments can be reduced with the help of sediment and sand traps, created as an extended meander and a special bank shape. The construction of this device is relatively simple, it is simply a widening and deepening of the drainage ditch or small inflow so that the flow at this point is reduced and sand and coarse organic material can settle and subsequently be easily removed (**Fig. 182**, **183**). To correctly design the sedimentation pool, it is necessary to know the flow

Fig. 179. Anchor ice in the water in a RRF with FPM.

Fig. 180. Trapping of eroded sediments by natural overflow. Natural overflows into harvested meadows in the lower part of Blanice NNM are very effective at capturing sludge and clay – the photo shows flooding in one such area during a minor springtime flood.

Fig. 181. A dried-out part of the floodplain which is regularly flooded; overflows do not prevent hay harvesting in the dry part of the year.

Fig. 182. Trapping eroded sediments in sedimentation pools. Sedimentation pool below the village of Zbytiny – a large amount of sand is apparent in the left of the picture, trapped by the pool during increased flow; on the right side, the main stream flows into the tall grass floodplain meadow where fine sediments settle (photo from 2012).

Fig. 183. The same pool after stabilization of the erosion damaged channel in the upper course of the river using growing fascine bundles; the water has returned to the original channel (photo from 2016).

velocity, flow rate, and the approximate amount of material carried by the flow during increased total precipitations. This measure reduces the movement of sand in the channel, especially during torrential rain, and acts as a prevention against excessive siltation of the channel. It is necessary to clear deposits from the structure at least once a year, but it is advisable to carry out regular checks after increased precipitation (Altmüller & Dettmer 2006, Simon et al. 2010b).

Regarding sedimentation pools on FPM streams, it should be noted that they are only temporary measures – they should only be needed until the direct source of erosion is removed. Capturing all sediments could eliminate one cause of threat to FPM (clay and sand) but also could accelerate another – trapping organic detritus which serves as food for FPM. Sedimentation pools should therefore be constructed to perform their function especially at increased flow rates, when the load is mainly mineral material. During normal flow, when organic detritus flows from food-producing wetlands and spring source areas, its capture is undesirable.

10.3.2 Soaking pools

These are small artificially created depressions, in which both fine and coarse sediments collect from ditches along roads. In contrast to sedimentation pools, they are lateral pools that do not flow directly into streams, and the water from them seeps into streams through the subsoil. The deposition of undesirable sediments is therefore very effective, even at high flow rates.

10.3.3 Semi-permeable dams

These barriers can also be one of the types of separating features, and they often perform multiple functions. Catchments with inappropriate agricultural or forestry management on steep slopes are often affected by erosion, where small watercourses were straightened and modified and drainage ditches were built.

In the case of large-scale erosion, during which gullies are formed and scouring occurs, it is first of all necessary to address the cause, namely to stabilize or restore the damaged channel. If overall restoration is not possible for some reason, or until the process is completed, one of the options to prevent excessive sediment from entering the stream is the construction of semi-permeable dams in critical parts of the river catchment (e.g., on tributaries or even in the main stream above the occurrence of FPM). It is necessary to recognise the hydrological conditions of the given river catchment and, if possible, to have an overview of how the relevant part of the river catchment behaves during high precipitation – where do rain rills lead and where does the suspended sediments flow? Only under these preconditions will it be possible to design the construction of semi-permeable dams in places where they will be really effective.

In oligotrophic catchments with FPM habitat, **it is not appropriate to use fibrous geotextiles and other non-natural materials for this purpose** (fine plastic fibres could be released and adversely affect aquatic animals that filter detritus from water, including FPM). Objects made of a large quantity of coniferous logs (with bark) and green branches from coniferous trees, which release phenolic substances in the aquatic environment, could also be problematic. The best material for this purpose are log fences, double willow or willow and alder wattle, filled with aggregate (**Fig. 184**), or stone ripraps or stone dams filled with soil or turf. Moreover, when willow rods are used they usually take root and, with appropriate insolation, they sprout, as well as when used on grates or for reinforcing embankments. The barrier is stable, functional, and aesthetic. Plastic or steel pipes on the stream bed can be used to create culverts through the dam (**Fig. 185**). Reinforcement is done with stone riprap and, under the pipe, the bed is sealed with compacted soil with a turfed riprap surface. A short pipe section will not degrade nutritious detritus, alter the temperature conditions in the flow, or impede migration (Dort & Hruška 2008).

Under normal water conditions, the dam is permeable, but if the flow increases, the pipe capacity determines the flow through it. The excess flow will rise and flow out in the specified direction. Here, it flows through a series of small arc-shaped dams, the flow velocity slows and thus the entrainment and erosive force of the current diminishes (**Fig 185**). The sand is then deposited on the floodplains (Vaníčková et al. 2016).

10.3.4. Minor erosion control measures

Minor erosion control measures include draining water back into the channel after unwanted overflows into natural small streams, caused by, for example, logging with heavy machinery in places where there is a risk of waterlogging of commercial forests or agricultural land. Furthermore, there is less extensive stabilization of the channels of small streams by stone ripraps, soil, or excavated turf which are left after creation of rills. It is possible to use willow-alder gratings, made up of rods left after pruning of these trees (Simon et al. 2010b). For more information on willow-alder gratings, see **Chapter 10.4.1.1**.

Small anti-erosion measures can also be described, in some cases, as minor non-structural restorations, such as manual modification of a small streams' direction, without changes to the property registration (so-called adjustment of the water conditions of the land). Most often, such modifications

Fig. 184. A dam formed by a double willow fence filled with quarry stone. At higher water levels, the water partially flows around the dam, partially through it. Both the fine-grained and coarse-grained suspended solids are trapped in front of the dam.

are implemented to prevent major intervention by the owner regarding waterlogging of forest, meadow, or track.

When draining water into the channel, and during manual modifications of small streams, it is recommended to do the work in dry conditions as much as possible, then gradually let water in, or take advantage of the possibility of draining the turbid water into the meadow vegetation (Řepa 2010).

10.3.5 Examples of specific measures for trapping erosion sediments

10.3.5.1 Large sedimentation pool on Zbytinský potok stream

Between 2004 and 2005, approximately 1,600 m of Zbytinský potok stream (right tributary of Blanice River) and Sviňovická strouha stream (right tributary of Zbytinský potok stream) were restored. During the restoration modifications, the original concrete channel was removed and the bed of the newly formed channel was modified using stone steps. Trees were planted to reinforce the bank. As a long-term monitoring of the stream has shown, the sediment regime was disrupted in the catchment before restoration, even though reducing the amount of sediment was one of the reasons for the modifications. The chosen restoration technique led to an increase in the amount of fine-grained sediments in the stream channel itself, mainly at times of higher flow rates. Tearing out the concrete lining of banks without water transfer had a significantly adverse effect. The flowing water immediately eroded the exposed stream bed and banks and scoured them. Before the start of the springtime thaw, the banks had not naturally stabilised, so the springtime water eroded them further.

Fig. 185. Semi-permeable dam. Water can flow through this barrier under normal water conditions; however, with increased flow the pipe capacity determines the flow volume. Excess flow rises behind the dam and overflows in the specified direction. To prevent the creation of a migration barrier for trout, the pipe must be placed so the water continuously freely flows through it without a hydraulic jump.

Before the restoration began, almost 6,000 adult FPM were in the section to be restored, with a very favourable distribution rate of individuals in the stream. There were three stable colonies which made up 92 % of the population. Two years after the restoration, only 80 % of the population was in colonies, which clearly indicates their collapse in search of a more suitable habitat. The 2006 census also confirmed increased mortality in the thousands of individuals (estimated mortality was 47 %) that either died under sediment deposits or were carried downstream where their biological requirements were no longer met (Spisar & Simon 2006, AOPK ČR 2007).

In 2005, the investor proceeded to take measures to reduce sediment input into the river and eventually eliminate it completely. As part of the restoration above where Zbytinský potok stream joins the Blanice River, a large sedimentation pool was created as a coarse sediment trap with a total volume of 15 m^3 (**Fig. 186, 187**). A stone filter was installed on the stream bed to allow permanent flooding. Most of the flow passed through this pool, where the entrained coarse material settled. Below the pool, an overflow into the eutrophic Filipendula grasslands was created, where the fine-grained particles were deposited and, at the same time, nutrients were partially removed (see **Chapters 10.5** and **10.6** for spill into vegetation, where it is introduced among the separating features; it is a multi-purpose measure). The importance of the construction of this pool has been well documented thanks to regular measurements of the content of suspended solids in the water (**Fig. 188**). At present, the channel of the Zbytinský potok stream is stabilized after restoration, the sedimentation pool is no longer functional, and the spill into the vegetation has been completely removed. The former pool is undergoing the early stages of wetland succession, with small areas of open water, and it is a relatively valuable habitat (**Fig. 182**).

Fig. 186. Restoration modifications above the mouth of Zbytinský potok stream where it enters the Blanice River. Large sedimentation pool at Zbytinský potok stream in 2005.

Fig. 187. A free spill into the tall grass floodplain meadow with *Phalaris arundinacea* and *Filipendula ulmaria* following after the sedimentation pool on Zbytinský potok stream (Fig. 186). The pool caught the sand and the spill caught part of the fine clay particles. Sediment from the pool had to be repeatedly removed using an excavator.

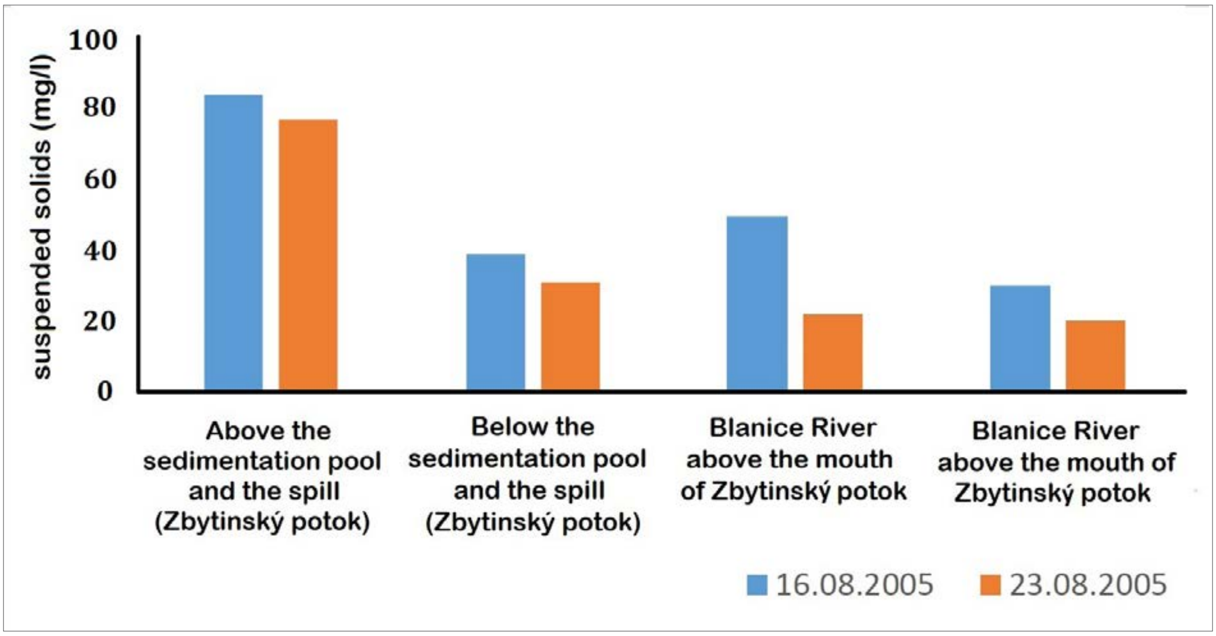

Fig. 188. Difference in the concentration of fine suspended solids (decrease in concentration) above and below the sedimentation pool and the spill on Zbytinský potok stream and the Blanice River above and below the mouth of Zbytinský potok stream. The effect of building of the pool and the sedimentation spill during the restoration of Zbytinský potok stream was well documented for two summer storms in 2005, when a significant decrease in the concentration of fine clay sediments in the stream was recorded. Sand transported on the stream bed is not captured by this measurement. Technically demanding measurement of the amount of sand has not been carried out anywhere in the Czech Republic yet, but it has been prepared on the tributaries of the Vltava River in Šumava National Park since 2018.

10.3.5.2 Sedimentation spills on the Luční potok stream

The spring source area of the Luční potok stream (right tributary of Zlatý potok stream above Miletínky Nature Reserve), the so-called Polučí, is drained by several tributaries of Luční potok stream and consists of a mosaic of harvested and non-harvested meadows, pastures, wetland forests, and wetlands (Simon et al. 2010a). At the same time, a deep gully has formed in the lower course of the stream due to erosion, which has not yet stabilized; due to the hydrological conditions and slope in some areas of the river catchment, it will be necessary to artificially stabilize the gully. During increased flow rates, a large amount of sediment was washed out of the eroded part of Luční potok stream (especially sand, fine-grained clay particles, and partly also gravel), which subsequently reached Zlatý potok stream, where it was deposited and degraded the FPM habitat.

In 2012, sedimentation spills were built (**Fig. 189–192**). There is a system of semi-flow-through partitions, overflows, and sedimentation areas on the stream which allow some of the water to flow over the bank during higher flow rates. By slowing down the flow through a series of side dams, both coarse and fine particles are deposited. The measure was implemented in two phases: non-structural on land managed by NCA CR and structural on land managed by the Czech State Forest (the state forestry organization). During the higher summer flows in 2012, the functionality of the system on NCA CR land was verified. During the first two years after the creation of the spills, approximately 80 m^3 of material was captured in this way at a distance of about 1 km from the spring source.

In 2015, the spills were modified on the basis of the experience gained during their operation; to allow water to enter side spill areas more efficiently, inlets were stabilised and retention capacity was restored and increased (AOPK ČR 2013a, Vaníčková et al. 2016). Currently, the restoration of the eroding section of Luční potok stream is planned; a feasibility study (Beleco 2016) was prepared. It should be emphasized that these are only temporary restoration measures until the overall restoration of erosion sites in the river catchment. Then the channel should be opened with full flow. However, it is desirable to keep the spills with dams in case they need to be used in the future (e.g., during construction works, road modifications, etc., where there is a risk of more sediment production). In this case, the channel can again be easily connected to the spill fields.

10.3.6 Monitored and evaluated parameters during anti-erosion measures

The implemented measures have been empirically verified as very effective. In the future, more accurate monitoring of their effect in measurable quantities will be required. In particular, it is necessary to measure physical and chemical parameters (conductivity, pH, content of nitrates, phosphates, and calcium) at the time of increased flow and turbidity, to monitor the amount of extracted material during the cleaning of sedimentation areas, and the inputs into the main flow resulting from the measure.

Published data are available, for example, from measurements above and below the sedimentation pool and spill at the village of Zbytiny. As part of the Action Plan (AOPK ČR 2013a), some parameters were measured in connection with sedimentation spills on Luční potok stream.

If a modified stream flows directly into a FPM stream above the occurrence of the pearl mussels or even above the colony, it is also advisable to carry out bioindication monitoring, for example by using Buddensiek plate which well indicates even short-term turbidity (see **Chapter 7** for more details).

Fig. 189. Technical implementation of measures on Luční potok stream in the Zlatý potok stream catchment. Spill just after completion of construction (a plastic pipe carrying the entire normal flow and use of coconut mats is visible).

Fig. 190. Measures on Luční potok stream in the Zlatý potok stream catchment during a flood.

Fig. 191. Measures on Luční potok stream in the Zlatý potok stream catchment after the high water level has fallen – coarse sand is deposited in the river channel, finer sediment on the sides (not visible).

Fig. 192. Similar location to Fig. 189 after the passage of several floods (followed by the cleaning of the pipe surroundings) and the return of dense vegetation.

10.4 Stabilization of erosion gullies and damaged channels

As mentioned in **Chapter 10.3**, the sediment inflow is a major problem for the survival of FPM. **Eroded potholes and gullies become long term sources of sediments, produced both by scouring power of water at high flow rates** and often also due to frost erosion of steep gullies or bare banks. Winter frosts can cause serious damage in damp places. When the thaw comes, whole blocks of soil slip into the channel. The soil is then transported by the flowing water. Quite severe frost erosion can also occur in the normal trapezoidal channels of surface forest drainage ditches that were excavated in the stream spring source sites, if their banks were penetrated by surface water. A larger network of drainage forest ditches in the spring source area can fill the entire flow profile of the brook at springtime so that the water rises to the bank edge which becomes waterlogged. The waterlogged banks gradually collapse, and this new supply of sediment is again transported by water to the main stream. Direct and subsequent erosion damage occurs on long stretches of watercourses with a long-term negative impact on the protected habitat and the population of FPM (Simon et al. 2010b). Therefore, in some cases, erosion gullies or channel sections above the FPM locations need to be either temporarily stabilized or appropriate measures taken to help natural stabilization (e.g., by rooting the banks).

10.4.1. Types of measures

10.4.1.1 Willow-alder grids

In order to restore eroded surface ditches and deepened parts of straightened channels, it is advisable to insert filtration features into the channel at a certain spacing, able to retain sediments, and at the same time gradually raise the stream bed.

In oligotrophic catchments with FPM habitat, **it is not possible to use plastic fibrous geotextiles** or **a large number of coniferous logs (with bark) and green branches of coniferous trees for this purpose** (for possible problems, see **Chapter 10.3.3**). Therefore, the best solution is to create **willow-alder grates**, combined with **stone ripraps**, as required (**Fig. 193**). The height and spacing of the willow gratings must be based on the slope so that the relative gradient is reduced, and there is good sediment deposition.

In the case of heavily eroded channels, it is advisable to restore the stream in several stages, combining the removal of excessively shading successional vegetation in the spring areas with use of material from tree pruning to stabilize the channels (Dort & Hruška 2008).

10.4.1.2 Partially flowing stone closure of gully

There are also frequent cases of erosion of an artificial channel excavated on a slope which, however, deviates in part or in the whole route from the natural thalweg of the original natural channel. In such a case, the best solution is to drain the water at a suitable location from the eroded channel into the natural thalweg and to introduce it into the original or newly established shallow channel (**Fig. 194**). At the same time, it is necessary to seal off the eroded channel with stone ripraps with possible use of gabion technologies with non-toxic anticorrosive treatment. Old stone drifts can be used for this purpose if they do not fulfil other important functions in the landscape. Stone closures of an otherwise dry channel are able to carry water during rainfall and melting snow but retain most of

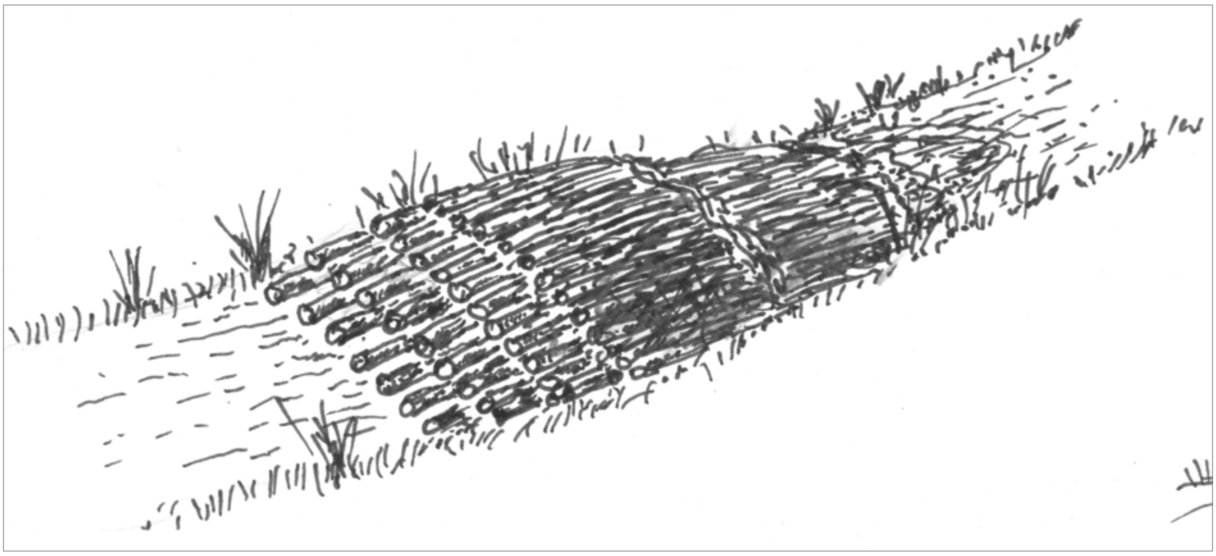

Fig. 193. Willow-alder grating – upstream side of the filter (right) with riprap of soil and stones. The riprap material is obtained by removing it from the steep walls of the gully. Illustration: Michal Bílý.

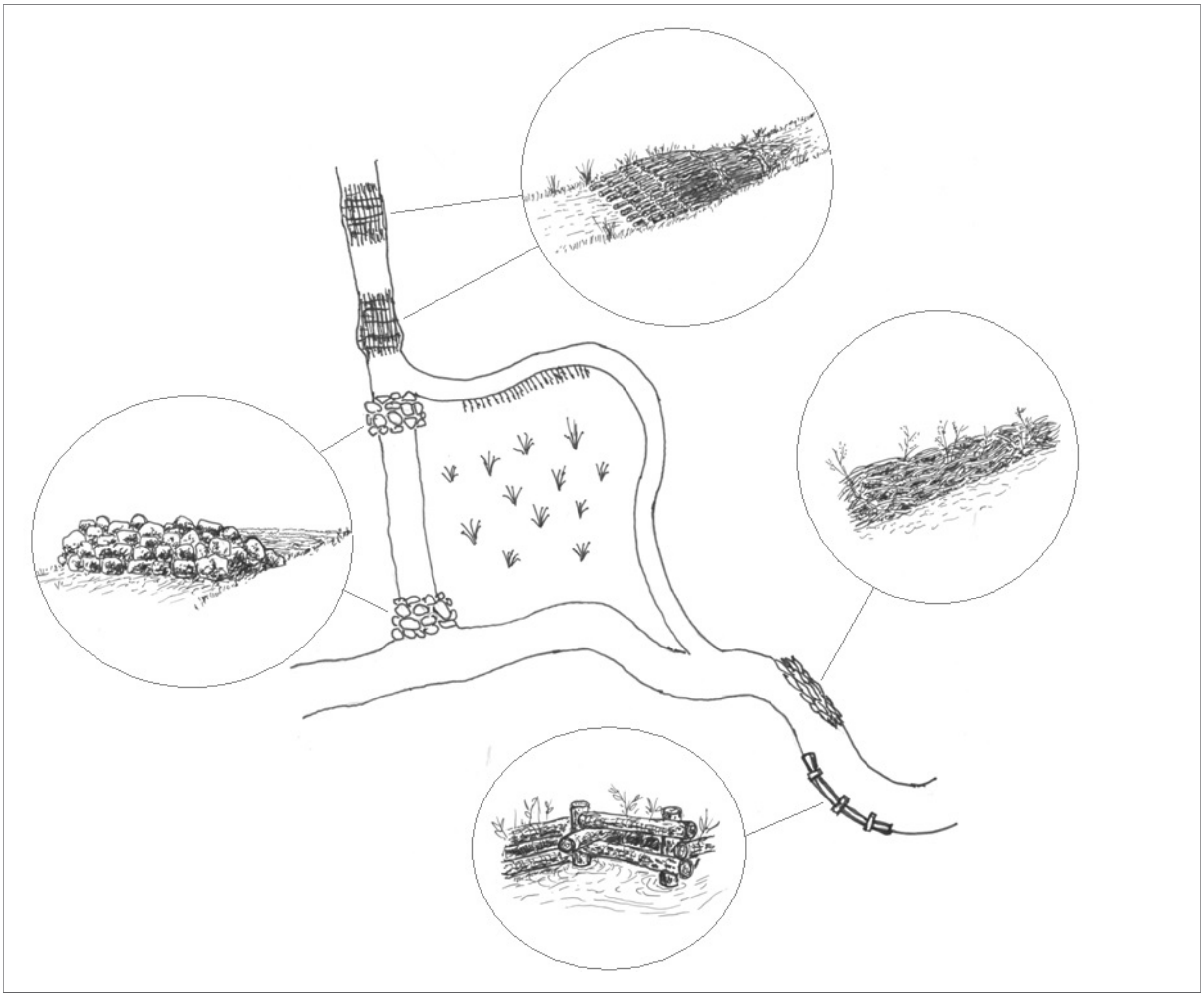

Fig. 194. An example of a combined measures for stabilization of an erosion damaged channel. Willow-alder gratings, partially flow-through stone closures and water outlet from eroded channel, fascine bundles, and log-wood restored willow construction. Illustration: Michal Bílý.

the sediment. Frost erosion gradually breaks down the steep slopes of gullies, gradual spontaneous restoration of the sloping of banks occurs and, once vegetation starts growing, natural consolidation of the soil takes place. It is also possible to drain water from the eroded channel into another nearby natural thalweg through a shallow channel. However, if this procedure is not possible and the water needs to be transferred via a longer route through an eroded channel, it is necessary to close at least the lower part of this channel, at a sufficient distance from the main stream, and divert the flow along the contour into another shallow channel. On the bank of this other channel, down the slope, a large enough spillway of higher flow rates into the vegetation filter is treated with stone, the efficiency of which can possibly be increased by creating several depressions into which sediments from the still insufficiently stabilized channel can be retained. Any such measures must be carried out on the basis of a detailed project and under the supervision of the relevant conservation authority (Dort & Hruška 2008). If possible, all works should be carried out in dry conditions, then water should be gradually introduced, and sludge sumps should be used (e.g., the possibility of discharging turbid water onto meadow grassland with small gravel pits; Řepa 2010).

The best way to create stone closures is by simply laying stones 'dry', that is, without using binding material, or using local clay or earth. Generally, the use of **cement mortar and similar materials in contact with water should be avoided** in FPM streams due to the leaching of calcium and the resulting increased loading of the FPM with this feature.

10.4.1.3 Growing fascine bundles with gravel

To help stabilize the eroded channels (**Fig. 195**), growing gravel fascine bundles usually serve well. In the case of larger damage, it is also possible to successfully use growing willow log constructions, installation of live trunks, or quarry stone fortifications (both animated and non-animated). These measures are described in more detail in **Chapter 10.5**. The term 'animated' is used in this

Fig. 195. Erosion, Blanice River.

context as containing live rods or trunks (especially willow) which, when adequately watered, root and grow, thereby stabilizing banks.

The growing fascine bundles with gravel (**Fig. 196**, **199**) are made of willow brushing with a gravel or stone filling. Willow rods can also be obtained from willows grown in rod gardens for this purpose. In small and large protected areas, however, it is always preferable to use willows that occur naturally in the locality. The diameter of the bundles is 800–1 000 mm. The brush packing has a thickness of at least 150–200 mm. The filling is made of river gravel with a maximum sand content of 20 %. The bundles can be made to any length. They are tightened by double wrapping with annealed wire 3–4 mm in diameter at 0.5 m intervals. The bundle ends must be closed by wattle so that the filling does not spill. In fast-flowing water, they are placed along pre-hammered stakes. It is advisable to create a bed made of a thick layer of branches under the bundles. The brushwood should be alive, from matured, not dried rods with undamaged bark and buds. Long, unbranched rods with a length of 2 m and a diameter of 20–40 mm are suitable for growing fascine bundles with gravel (and possibly other measures with the use of willow rods and wattle of such as wattle fences, willow coverings and fascine bundles). Shorter rods can be used in animated cribworks, rockfills, and ripraps (TNV 75 2103).

10.4.2 Examples of specific implemented measures, their description, and function

10.4.2.1 Growing fascine bundles with gravel on Zbytinský potok stream below the village of Zbytiny

As a result of the inappropriate restoration of Zbytinský potok stream and Sviňovická strouha stream in autumn 2004 (see **Chapter 10.1.3.1**), massive lateral erosion of Zbytinský potok stream channel occurred in the years following it (**Fig. 197** and **198**). In addition to measures to prevent sediment from entering the main stream of the Blanice River, in March 2006 it was proposed to reinforce the most critical sites with fascine bundles to alleviate lateral erosion. In the following years, the eroded banks were completely strengthened thanks to this measure and, at present, there is no longer any obvious difference between the natural young bank vegetation and the artificial reinforcement of the damaged channel with fascine bundles. After ten years, however, it should be noted that the young riparian stands were no longer being treated following the measures and it would be appropriate to prune the willow stand and remove the shoots growing into the flow profile. The live material collected would then be suitable for use on other growing fascine bundles with gravel. Detailed photo-documentation of the whole restoration development by the fascine bundles on the Zbytinský potok stream is given in **Annex 14**.

10.4.2.2 Monitoring of vegetation features

Fascine bundles do not need any maintenance in the first years after installation. The erosion control effect is immediately noticeable and, with rooting and growth, a riparian willow stand gradually arises. It is necessary to check the site after 3–6 years. Most bank bundles do not need any maintenance (**Fig. 200**), but pruning is possible to obtain brushwood for further use. If some shoots grow into the flow profile at normal flow rates, they must be cut back (**Fig. 202**, **203**). In most cases, the lower shoots die off due to shading and the channel self-cleans (**Fig. 201**).

Fig. 196. Zbytinský potok stream in the village of Zbytiny – reinforcement of the eroded channel with a fascine bundle. Growth on the fascine bundle after the first growing season (for details, see Annex 14).

Fig. 197. Restoration process on Zbytinský potok stream and Sviňovická strouha stream. Removing of concrete panels and leaving of a not naturally deep channel on Sviňovická strouha stream in autumn 2004 led to excessive entry of sediment runoff into the main stretch of the Blanice River (a FPM location).

Fig. 198. Erosion of a broad channel with not natural volume due to storm flows in August 2005 (Zbytinský potok stream).

Fig. 199. One metre high ongoing erosion stabilized by a growing fascine bundle in November 2006 (Zbytinský potok stream).

When setting up new bundles, close supervision is required to ensure that live wattle from willow species is used which can continue to grow at the site. If necessary, only 50 % of live brush is sufficient.

10.5 Stabilization of valuable meanders

Historically, meandering watercourses have been a common feature of the landscape. The natural dynamics of meandering flows in wide valley floodplains lies in the process of deepening, breaking, and creating new meanders. Other natural processes, such as the channel creating activity of the main stream or erosion of natural stream channels of the tributaries, are very important for the existence of the flowing environment of the riverbed and directly create this habitat. Given that the FPM streams are usually found in small or large Protected Areas (PAs), these natural processes should logically be „green" and the breakage of meanders should continue to be a natural part of the development of the river landscape.

Given that the main subject of protection in these protected areas is the freshwater pearl mussel, the interest of **protection of this species may take precedence over the protection of natural river dynamics in some cases**. Therefore, in justified cases, specific endangered meanders are stabilized by appropriate procedures. These may include, for example, **an unstable valuable meander above the colony of adult FPM, retaining meanders for individuals involuntarily flushed by the stream, stretches of stream in the reproductive part of the habitat, where it is expedient**

Fig. 200. Example of maintenance-free fascine bundles. Condition of fascine bundle after 10 years (800 m above sea level), clear flow profile, young trunks are growing upright.

Fig. 201. Low-lying branches are already dry; intervention is not necessary.

Fig. 202. Example of maintenance-free fascine bundles. The horizontal trunks reaching into the flow profile must be cut.

Fig. 203. Detail of a site where a fascine bundle was placed – the erosion control function was taken over by the dense riparian stand of rooted willow rods (*Salix fragilis*).

to create suitable locations for occupancy of host fish by glochidia (parasitic stages of young pearl mussel), or other reasons, especially when it comes to e.g. border flows.

10.5.1 Options for stabilizing meanders

Stabilizing of a valuable meander that is at risk of breaking usually has a relatively large impact on the flow and is preceded by the processing of project documentation. Cooperation is essential between the respective flow administrator and the nature conservation authority (which has an overview of endangered FPM colonies, ensures regular field investigations and long-term management of the site, and issues the necessary exceptions to the basic conservation conditions of specially protected species). As far as the work in the channel itself and the bank line is concerned, it is not necessary to deal with the modifications with the owners of the surrounding land, except to ensure access to the meander and the entry of the equipment. However, if stabilization of the meander requires a temporary transfer of water, an agreement with landowners is necessary. As with other interventions directly in a channel above FPM, care should be taken not to increase the sediment input into the stream too much, in particular to avoid the movement of equipment across the main stream.

Therefore, whenever possible, it is better to prevent the problem with more appropriate and ongoing management of the riparian stand. On streams (depending on their size), these are usually small-scale and less complex interventions (unless it is a border stream).

10.5.1.1 Live willow log structures

Single-walled log cribworks (**Fig. 204**, **205**) consist of longitudinal logs with a diameter of 100–300 mm, attached to stilts with a diameter of 160–180 mm, and anchored behind the wall. The stilts are connected to the longitudinal logs at joints. The wall face generally has a 5:1 slope. Local willow logs should be used. The inner space behind the log wall is filled with a local quarry stone (a certificate for leaching of Ca and toxic elements is required!); the best-case scenario is stone obtained directly on site. Wedge stones are placed behind the longitudinal logs with a wider dimension against the river bank. The crown of the wall is made of selected stones wedged against each other (modified according to TNV 75 2103). On smaller streams, a reasonably simplified structure can be used.

10.5.1.2 Installation of live trunks

Semi-uprooted willows with a growth of new tree branches are an excellent live vegetation feature for stabilizing the impact banks of meandering streams in locations where natural flow dynamics are undesirable. The trunks are firmly seated and pegged to the bottom of the bank, and their thick branches are pushed sufficiently deep into the bank.

Long willow rods with a thickness of 3–5 cm are also used as vegetation treatment. Holes to a depth of 0.5–1 m are initially made with steel punches perpendicular to the bank at the level of normal flow; the thicker end of the pointed live willow rods are pushed into the holes. Then, after being bent upwards, the rods are pushed into inclined openings, again formed by the punch in zigzag pattern in a strip 1 m wide, but at least 0.5 m from the edge of the bank. Willow rods usually take root at both ends, with the new branches sprouting from the bends. Over time, part of these new rods should be bent again and planted into the bank. Thus, a strongly-rooted overhanging bank can be created that can withstand high flow rates. In places with a stronger flow, it is advisable to place large

Fig. 204. Living willow log structures. Close-up view of a growing willow log structure in early spring-time – willow trunks interspersed with quarry stone.

Fig. 205. Overall view of the willow tree structure after one vegetation season.

stones at the foot of the bank or to use a stronger trunk well attached to the stream bed and bank (Simon et al. 2010b).

10.5.1.3 Quarry stone fortifications (animated and non-animated)

Stone scatter and riprap is the most common way of strengthening the banks of watercourses in the Czech Republic (**Fig. 206**). Stone scatter consists of stones weighing up to 200 kg; boulders weighing 200 kg or more are used for stone riprap. Stone riprap stabilizes banks with a steep slope. Often, stone riprap is used only at the foot of the slope, while higher up the slope, above the water surface, the bank is reinforced with stone scatter. In oligotrophic catchments with FPM, it is always necessary to use a local stone, which does not risk harmful leaching in these waters with very low conductivity. **Unanimated riprap or scatter is not very suitable in FPM streams; regeneration of banks to a near-natural state takes a very long time, the banks are not rooted by riparian vegetation and thus do not provide suitable shelters for host fish.** Details of technical design are set by ČSN 72 1800, ČSN 72 1860, and ČSN 72 1151. The scatter can be made from river pebbles, cobbles, or other materials, always strictly of local origin.

An animated riprap is created similarly to non-animated, except that the gaps between the stones are filled with gravel and the riprap is inserted perpendicular to the slope with willow rods (bundles of willow rods) with a minimum length of 0.5 m. The thicker rod ends must point into a permanently wet bank layer. The diameter of the rods is 30–40 mm. It is preferable to deposit the riprap on the willow bedding, then it is not necessary to interleave it with the rods perpendicular to the slope. When creating an animated scatter, the scatter is deposited on the bedding of the brush (modified according to TNV 75 2103). In contrast to the riprap and scatter, which necessarily requires heavy machinery,

Fig. 206. Loose quarry stone meander reinforcement, created for the necessary stabilization of the bank above the transverse stage irrigating the side branch at the Spálenec RRF.

it is usually preferable to use in situ assembled growing fascine bundle with gravel in catchment areas of FPM in small and medium streams (see **Chapter 10.4.1.3**). It is always essential to work correctly with the live brush, which must be carefully checked.

10.5.1.4 Clearing obstacles from waterways and meanders

A preventive measure against damage to valuable meanders and problematic parts of a stream is the removal of obstacles and, in general, the clearing of streams in places where floating wood and other material accumulate in the channel. The work should be carried out manually or with the aid of light machinery (**Fig. 207**, **208** and **209**).

As with meander stabilization, it is necessary to prevent these situations if possible and to remove the key elements of future obstacle (usually large trunks) before sealing the barrier with a finer material. This will also prevent large accumulation of fine sediments upstream of the obstacle. However, preventive measures are often not always possible for obstacles created accidentally during a flood. This can be prevented by the selective management of dead wood in the upstream section, for example removing or retarding transportable trunks and their parts, as described in the methodology of Kožený et al. (2011, 2012).

Fig. 207. Example of making watercourses passable. Removing an obstacle threatening a valuable meander above Spálenec RRF centre.

Fig. 208. Exposed sediments after removal of obstacle.

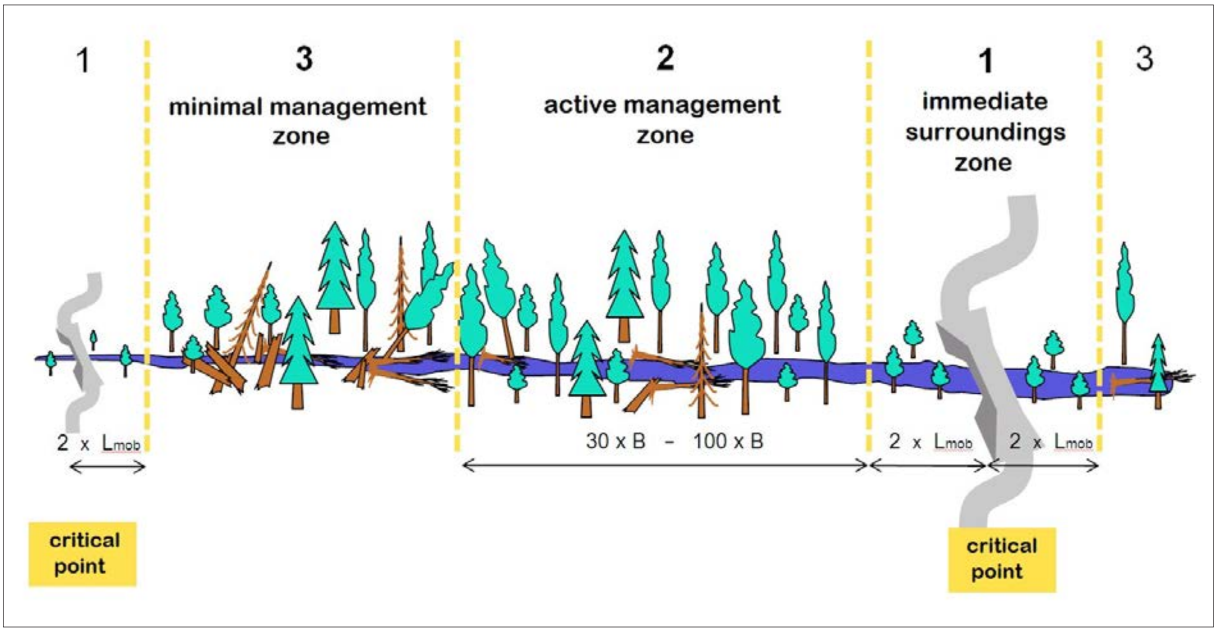

Fig. 209. Diagram showing selective management to prevent formation of barriers (a critical point may be a bridge or a flow narrowing), after Kožený et al. (2012).

10.5.2 Examples of specific implemented measures, their description and function

10.5.2.1 Strengthening the Blanice River meander under power transmission line

In 2009, technical measures were carried out to stabilize the meander below the power transmission line in the main the Blanice River; bank erosion after the passage of flood threatened a stable colony of FPM. However, the stabilization structures had to be maintained repeatedly; for example, part of the obstacle was dismantled because of higher flows in 2012, so in 2013 the Blanice River meander was consolidated and the relief channel was cleared (**Fig. 210**; Simon et al. 2010b, AOPK CR 2014). The meander was already stabilized after three years of implementation (**Fig. 211**). Unfortunately, most willow cuttings which should have reinforced the bank with a root system did not take (**Fig. 212**); it is always better to use willow bedding (see the text above). In the future, therefore, it would be good to see if at least spontaneously established willows have taken hold. The lack of riparian stand and sandy banks could cause problems with excessive sediment input and repeated bank disruption during floods.

10.5.2.2 Strengthening Blanice River meander below the mouth of Tetřívčí potok stream

Further strengthening of a critical meander was carried out in 2014 below the mouth of Tetřívčí potok stream. In 2010, the old seal had already begun to open where a feature/rill was once experimentally built. The leakage gradually increased until the river relocated itself into the rill in spring 2011 (AOPK ČR 2013b). In 2012, a rescue transfer of FPM from the original bed of the Blanice River had to be carried out.

In 2014, the meander was repaired in cooperation with NCA CR and State Enterprise Povodí Vltavy; because heavy machinery had to be used, the riparian vegetation was cleared. The original meander was reinforced by a massive stone riprap and the new, shortened channel at the location of the trial rill was closed (**Fig. 213**, **214**). Subsequently, new trees were planted. In this case, small long-term preventive management would probably have prevented the need for such a costly stabilization structure.

10.5.2.3 Blanice River main stream – meander repair near the village of Arnoštov

In order to reduce adverse erosion incurred during flooding in 2002, the breach in the bank of the Blanice River meander was repaired in 2012. The breach came within almost three metres from the former millrace, full of muddy, strongly eutrophic sediments. If the millrace was breached, sediments containing hydrogen sulphide and other pollutants would escape into the river. The bank was reinforced with fascine bundles covered with soil and graded. The most exposed part of the bank was subsequently reinforced with mats (AOPK ČR 2013b).

Fig. 210. Meander strengthening below the power transmission line on the main stream of the Blanice River – location and extent of the works. Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

Fig. 211. Meander strengthening below the power transmission line on the main stream of the Blanice River above the FPM colony. View of the stabilized meander (springtime 2016).

Fig. 212. At present, the meander is generally stable, although most willow cuttings have not taken hold.

Fig. 213. Reinforcement of the Blanice River meander below the mouth of Tetřívčí potok stream. Photo from 2012 before strengthening. Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

Fig. 214. Photo of the same meander from 2015 following strengthening. Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

10.5.3 Parameters monitored and evaluated in connection with meander stabilization

Given that meanders are often strengthened by extensive construction, **basic physical and chemical parameters of the water (including temperature, conductivity and, if necessary, turbidity and nitrate load) should be monitored** during the implementation of the measure. It should also be remembered that modifications mostly take place on the main stream above the FPM, so that **direct effects of the work can be expected on them and their habitat.** Measurement of physical and chemical parameters, preferably continuous (e.g., using a continuous conductivity probe), above and below the site of intervention, may alert to potentially unsuitable materials such as aggregate with a higher calcium content than its natural in the FPM water flow. With continuous conductivity measurement, it is also possible to record breaches of technological rules, for example, when the mechanization moves in or near the flow. Valuable meanders are inherently found in places with or above FPM, so it is advisable to carry **out bioindication monitoring using juveniles** during construction work. Possible procedures for bioindications are listed in **Chapter 7** and a basic overview of bioindication tests is given in **Annex 15**. For the same reason, it is necessary to regularly inspect the pearl mussels that occur below the implementation site at regular intervals during the work.

When the work is completed, it is advisable to check the status of the restored flow and the amount of sediment carried by the flow during regular walkdowns (and also after heavy rains and floods). Mostly, these are very exposed sites in terms of scouring. Erosion may reoccur if the meander is not yet stabilized by the roots of bank vegetation and, at the same time, the materials used for temporary reinforcement (e.g., jute geotextiles) have begun to decay. If willow rods are used, they usually take root quickly, but this is not always the rule (see **Chapter 10.4.2.1**). Large ripraps and scatters of quarry stones are relatively durable, so they can be checked at random.

10.6 Food, warming, and separating features

Food and warming features (**Fig. 215**, **216**) **serve to increase the interaction of water and wetland communities, compensating for the adverse impact of technical modifications in river catchments and afforestation of hitherto open areas.** In both cases, it is restoration of a strongly structured watercourse in the form of a small stream, side branch, or shallow spill. These features are designed according to local morphological and pedological conditions to minimize erosion processes. Piped sections of watercourses are replaced by a shallow channel with winding of the channel (deep, permanently flooded parts of the flow) depending on the slope of the terrain. **Most of the restored natural water features are both warming and feeding** due to the occurrence of spring source vegetation and fauna, which increase food detritus input into the river network (Simon et al. 2010b).

10.6.1 Food rills and structured channels

So-called food rills in floodplains contribute to the interaction of the grassland root system with the aquatic environment and promote the connection between shallow groundwater and surface water (**Fig. 171**, **215**, **216**).

In places where the fine hydrographic network has been damaged by deepening, straightening, or piping, it is possible to restore it by creating a new shallow channel, which is gradually and

Fig. 215. Example of warming and food feature. Warming spill with a growth of *Scirpus sylvaticus*.

Fig. 216. A food feature with a varied composition of wetland vegetation on Blanice River.

spontaneously formed into the desired shape by water flow. When establishing a new channel, it is necessary to maintain the integrity of the rooting turf for the location of future banks; therefore, it **cannot be done by mechanical excavators** that tear the turf and do not produce good turf clumps, but only manually with a sharp turf spade. Using a sharp turf spade, the lines of both banks are cut and, then, the turf clumps are transversely cut out and brought onto the turf stowing. The turf in stowing is then treated and composted to obtain calcium-enriched soil.

The channel profile is square. The width and depth of the channel is a uniform 25 cm, regardless of flow rate (up to 15 l/s) (**Fig. 217–220**). Considering the nature of the terrain, **it is desirable to create numerous counter-directional arcs**. The channel slope should be in the range of 0.5 to 0.7 %. If the route is appropriately meandered, the method can be used on slopes up to 1.5 %. Flowing water then forms the channel into the desired shape. A channel of these dimensions is able of transfer a flow rate of 10 to 15 l/s. **During spontaneous shaping of the channel, sediments are formed which should not be allowed to descend into the main stream. Sedimentation spills into the turf are set up on the channel to help them settle.** Below these spills, the water is captured again into a rill. Flows through wetlands with favourable vegetation composition (*Cardamine amara*, *Stellaria alsine*, *Chaerophyllum hirsutum*, etc.) are formed at sedimentation spill sites, which are also involved in the formation of nutritive detritus for juvenile stage pearl mussels. After the natural completion of the channel, it is necessary to decide, depending on the local situation, whether it is appropriate to maintain the spillway or connect the channels. **This type of channel is suitable for meadow thalweg streams, where the water is already sufficiently warmed at a certain distance from the spring source.** The shape of the channel and the riparian vegetation no longer allow further warming of the water by the sun; temperature conditions are influenced mainly by soil temperature and the rate of infiltration through the soil environment. **Channels of this type can significantly improve the production of detritus from the rhizosphere of the bank vegetation**. Channels created by larger soil-living animals (mainly *Arvicola amphibious*), and other channels in the soil, also create a concentrated water flow to the wider surroundings of the stream, producing a mosaic of small wetland habitats. Detailed channel tracing and spill implementation must be defined in the terrain according to site conditions (Dort & Hruška 2008).

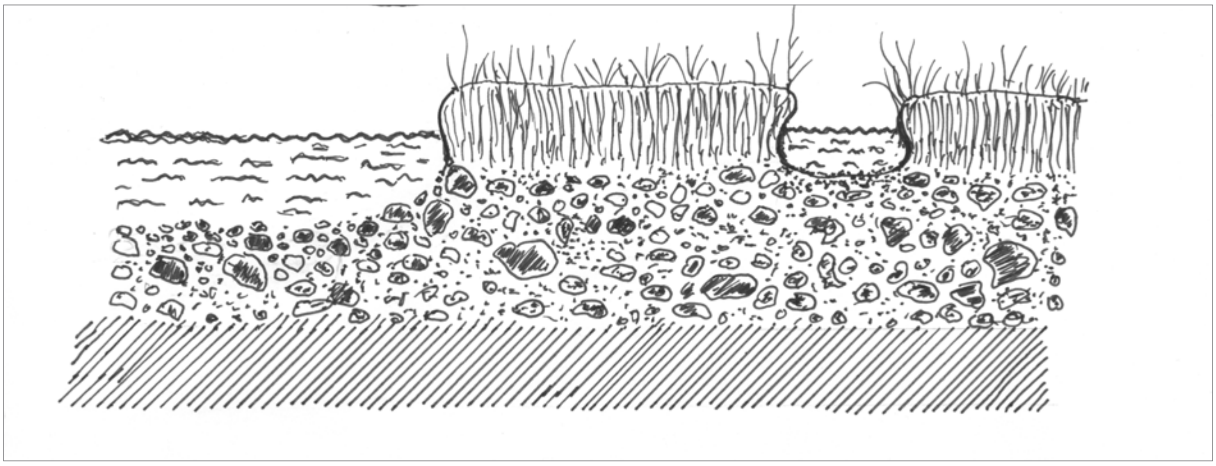

Fig. 217. Structure of a food rill. Illustration of a food rill showing permeable and impermeable subsoil – in the permeable subsoil, water from the main stream interacts with water in the food rill; dead grass roots create detritus (i.e., food) for the FPM. At the same time, the aquatic environment warms up in the food rills and in the whole of the waterlogged floodplain. Illustration: Michal Bílý.

Fig. 218. The riparian structure of the rills in the wetland vegetation is very varied, which makes a large wetted surface – there are wider sections (far left), areas with staggered banks, and places where the turf grows completely over the channel (far right) – ZRRF. If one walks carelessly, the banks can easily be stepped through (in the middle).

Fig. 219. Example of a food rill. Food rills should be checked regularly and restored to their original width every few years because they gradually become clogged and overgrown. Cleaned food rill in the Lužní potok stream catchment.

The term structured channel means a small meandering flow of first to third order (according to the Strahler stream order) with a large wetted surface and grassland with mowing or composting management. Structured channels have many advantages: the production of detritus, which goes into water; a habitat for detritus transforming aquatic organisms; influence on water temperature; stabilization of runoff into a non-eroding, less steep channel. Unlike food rills, these are usually natural streams or channels artificially created but currently functioning as natural streams. In some cases, these channels may have a suitable habitat for juvenile FPM, which is not the case with food rills.

10.6.2 Flow through and non-flow through pools

Built as food and warming features, these pools are complex habitats where water is heated, and food detritus is transformed. On the routes of meandering channels, it is advisable to create small side pools which also act as refugia for protected species (shelter from unfavourable conditions). These pools represent substitute habitats for the gradually filling pools created in landscape free of human settlement by the natural shift of meanders of the river.

An example is the lateral biological and sediment flow through pool at ZRRF (**Fig. 176**, **221**, **222**), which was built as biological one, but at the same time traps sand in the first part of the rearing feature. This created a complex habitat that transforms detritus on the rest of the area and also fulfils a warming function.

Fig. 220. Under dense vegetation, the rills are only visible in the non-vegetation period. The rills at 'pod Bělákovou' in the Sněžný potok stream catchment, the mowed strip and the tussocky uncut vegetation of the wet meadow are clearly visible.

10.6.3 Separating features – spill into vegetation and eutrophic water seepage

Separating features are defined as post-treatment cleaning spillways and flow-through wetlands, the wetland vegetation of which is able to effectively extract nutrients brought in from higher-lying pastures or fields and convert them into organogenic detritus. Extra nutrients can come from both human activities (especially pastures, fields, or settlements) as well as from natural geological subsoil with a higher mineral content.

Flow-through wetlands are created by rills and small spills and must have high insolation. Subsequently, at the lower part of the feature, the water must be returned to the channel by means of a collecting rill. Purification wetlands can be either natural (i.e., water with increased nutrient content is drained into an existing wetland), or artificial, where a semi-natural gravel filter is created, planted with wetland vegetation. Gravel ripraps and stone weirs can be used to create flow-through wetlands and spills (Simon et al. 2010a).

Proper functioning of infiltration pools can be supported by planting suitable wetland vegetation. Wetland plants have the ability to significantly adapt to permanent or periodic flooding, lack of oxygen, high salt content, and sudden changes in pH. In principle, plants suitable for a infiltration pool are those used in wastewater cleaning stations (*Phragmites australis*, *Typha latifolia*, *Typha angustifolia* – narrowleaf cattail, *Glyceria maxima* – reed sweet-grass, *Phalaris arundinacea*, *Schoenoplectus lacustris* – common club-rush, *Sparganium erectum* – branched bur-reed, and *Juncus effusus*), while the nutrients load in the infiltration pool can be significantly reduced. If the infiltration pool is outside an urban area in a small or large protected area, it is always necessary to start from planting the infiltration pool with naturally occurring vegetation in the locality, or to abandon planting completely and let the pool develop spontaneously.

10.6.4 Examples of specific implemented measures, their description and function

10.6.4.1 Food and warming features, structured channels

These small features were implemented in various modifications in many places in practically all catchments with FPM. Typical examples are: Spálenec RRF centre, Spálenecký potok stream catchment, Sněžný potok catchment (left tributary of the Blanice River below the village of Spálenec, in the local section “pod Bělákovou”), Zachráněný potok stream in the village of Miletínky, rills at LRRF, restoration of Lužní potok stream tributary in the village of Pastviny, etc.

10.6.4.2 Spill below the village of Miletínky

Below the village of Miletínky, features were built separating eutrophic water from the built-up area of the municipality. These waters drain into a natural after-treatment wetland or a semi-natural gravel filter planted with wetland vegetation. The dissolved nutrients are incorporated into the biomass of emerging (above surface) vegetation (**Fig. 222**).

Fig. 221. Example of a side pool. The flow pool in the Zlatý potok stream catchment (ZRRF) with a combined function; it was built as an experimental-model feature to test expected functions. Pool immediately after excavation in 2010.

Fig. 222. The same pool with macrophyte vegetation; by producing organic detritus, they maintain a good food supply for FPM.

Fig. 223. Infiltration (sedimentation) pool under the notch of the road through Miletínky NR (springtime 2016).

Fig. 224. Shallow infiltration ditch in Miletínky NR during a dry early springtime.

Fig. 225. Detail of the final periodically flooded part of this ditch, with emergent wetland soft vegetation (view in the opposite direction).

10.6.4.3. Soaking pool below the notch of the road through Miletínky NR

The roadside ditch leading water from the road leading steeply up through Miletínky NR originally led directly into the watercourse. Thus, there was runoff of clay turbidity and dissolved substances from minerally rich serpentinite subsoil into the lower part of Zachráněný potok stream. The drainage of these waters into a sedimentation pool with a spillway into the wetland, built in cooperation with the forest owner, has proven to be successful (**Fig. 223–225**; Simon et al. 2010a).

10.6.5 Parameters monitored and evaluated in connection with food, warming, and separating features

All the above-mentioned small features are usually created manually or using light machinery; however, during construction it is necessary to monitor the sediment regime and possible influence on FPM. If precipitation increases significantly during the work, it is advisable to monitor the sediment regime, including a one-off measurement of physical and chemical parameters, or to identify locations with increased erosion which will need to be better secured.

Procedures that increase the mineralization of introduced organic detritus (e.g., intensive aeration of water at weirs) should be avoided during minor flow adjustments on all tributaries that are important for the food supply of freshwater pearl mussel habitat.

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List of abbreviations

AFDM – Ash-free dry mass
CAP FPM – Czech Action Plan for Freshwater Pearl Mussel (AOPK ČR 2013a)
CPOM – Coarse particulate organic matter
ČSN – Czech technical standard
D° – The sum of the average daily water temperatures
DDG – Gravity-type sampler of detritus in running waters providing a continuous sample flow (Patent EP-2128595)
DDP – Pressure-type sampler of detritus in running waters providing a continuous sample flow (Patent CZ 303836)
FBA – The Freshwater Biological Association
FPM – Freshwater pearl mussel
FPOM – Fine particulate organic matter (0.5 µm – 1 mm)
GF/C filters – Whatman glass microfiber filters, Grade GF/C
GSM – The Global System for Mobile Communications
HRCZ – The Habitat Catalogue of the Czech Republic (Chytrý et al. 2010)
LRRF – The rearing and reproductive feature at Lužní potok stream
LU – Livestock unit
LWD – Large wood debris
NCA CR – Nature Conservation Agency of the Czech Republic
NM – Natural monument
NNM – National natural monument
NP – National park
NR – Nature reserve
PA – Protected area
PET – Polyethylene terephthalate
PIT – Passive Integrated Transponders
RRF – Rearing and reproduction feature
SAC – Special Area of Conservation, NATURA 2000
SCI – Site of Community Importance, NATURA 2000
SPA – Specially Protected Area
Spálenec RRF centre – A smaller pilot rearing and reproductive feature built in the original branch of the Blanice River at the village of Spálenec
SRM – Summary of Recommended Measures for Special Areas of Conservation according to the Government Decree No. 114/1992 Coll.
SRRF – The rearing and reproductive feature at Spálenecký potok stream
TGM WRI – T. G. Masaryk Water Research Institute
WWTP – Wastewater treatment plant
ZRRF – The rearing and reproductive feature at Zlatý potok stream
0+ – First age cohort: pearl mussel individuals of the first growth period (from the time they fall off the fish)
1+ – Second age cohort: pearl mussel individuals after the end of the first growth period, i.e. in the second growth period (we are not talking about the age of 1 or 2 years, because with semi-natural rearing it is possible to „make it“ two growth periods in the first calendar year by shortening the winter rest period - for details, see the rearing chapters)

2+ and so on – Further age cohorts, see 1+
3MAX – The 3 largest individuals (the most growing individuals)
10MAX – The 10 largest individuals (the most growing individuals)

List of annexes

Annex 1. Water purity limit values for freshwater pearl mussel occurrence

Table 4. Water purity limit values for FPM occurrence; after Absolon & Hruška (1999), unless stated otherwise.

Environmental parameter	Limit value
Nitrates	< 2.5 mg/l NO_3
Phosphates	< 0.3 µg/l (Young 2005 according to Oliver 2000)
Total phosphorus	< 20–35 µg/l
Ammonia (NH_4^+)	< 0.1 mg/l
pH	6–7.1
Conductivity	< 70 µS/cm
Max. temperature	20 °C
Calcium	< 8 mg/l
Soluble organic substances	< 1.3 mg/l (Young 2005 according to Oliver 2000)

Total phosphorus in water consists of phosphates as the main component of dissolved (total) reactive phosphorus (in addition to polyphosphates and some easily degradable phosphorus-containing organic substances, e.g. ATP), as well as bacterial and phytoplankton cells and detritus.

Annex 2. Water temperature limit values for freshwater pearl mussel occurrence

For a successful reproduction cycle, it is necessary, among other things, to ensure the optimal course of the temperature curve of water. As FPM currently find suitable habitats only in the colder streams of the foothills, it is desirable for the water temperature to exceed 15 °C for a certain part of the year. Only then does the glochidia mature inside the females and the larvae are subsequently released into the aquatic environment. The influence of temperature regime on reproduction is described in detail by Hruška (1992a). Bauer & Wächtler (2001) observed the highest rate of release by the female pearl mussel at the time of maximum daily temperature. The second condition for the continuation of the reproductive cycle is reaching the daily temperature necessary for the successful course of metamorphosis on the gills of the host fish.

Due to low temperature, other negative phenomena occur. A serious problem in this case is the reduction of the nutritional value of organic detritus. With the temperature dropping, its decomposition rate decreases and thus its use by FPM is reduced (Hruška 2004). On the other hand, a permanently higher temperature accelerates the metabolic activity of bivalves, increases their growth, and thus shortens the life span of individuals down to 40 years of age (Ziuganov et al. 1994).

The maximum temperature of 25 °C, which is the limit for the species according to Degerman et al. (2009), or 20 °C according to Absolon & Hruška (1999) is not usually achieved in Czech watercourses with the occurrence of FPM (**Fig. 226**). An exception are some sections of Blanice River with a wide, shallow riverbed, where the riparian vegetation died of graphiosis. In July 2010, for example, the water temperature rose repeatedly above 23 °C with a large daily amplitude, with lows below 16 °C (J. Hruška, unpublished data). In the exceptionally warm year of 2015, the rare maximum temperature of 25.5 °C was recorded, with the July average of 17.7 °C (Černá et al. 2017).

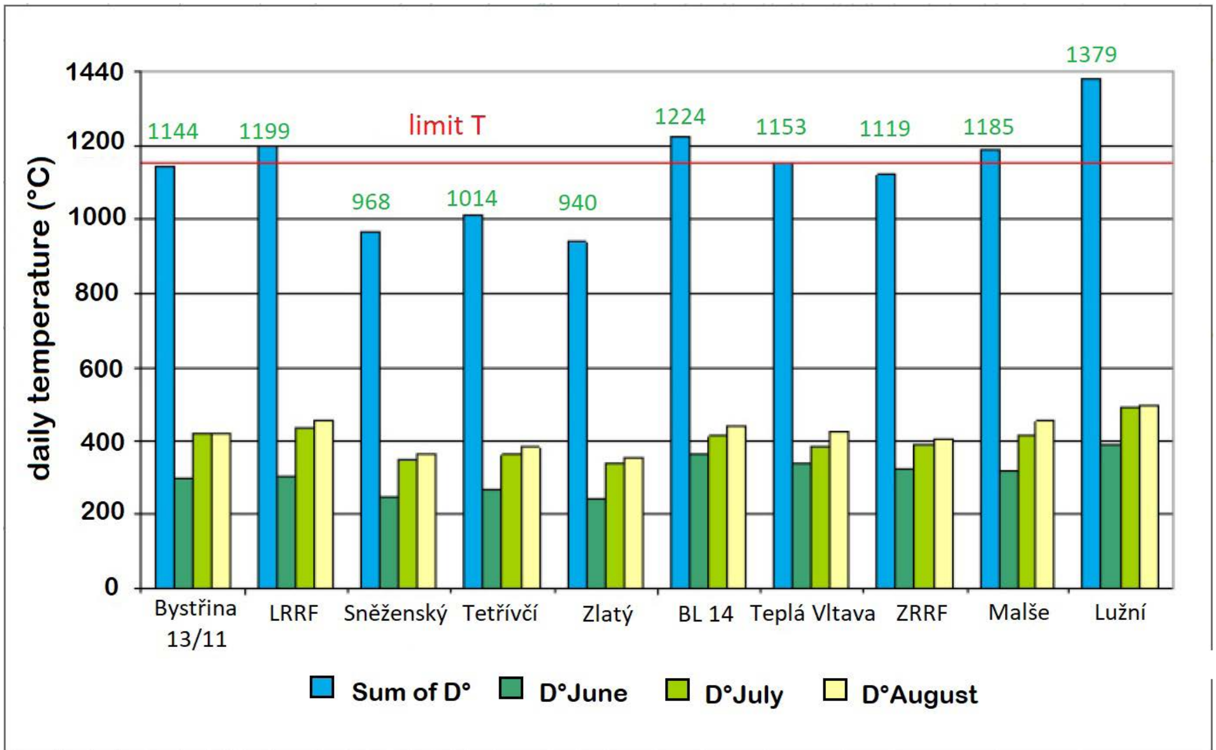


Fig. 226. Achieved sum of daily temperatures in selected FPM sites. Red line shows temperature limit for FPM reproduction. Data: NCA CR.

Annex 3. Influence of land-use on water chemical composition

In 2016, area sampling of FPM river basins and their immediate surroundings was performed in order to document the current state of water chemical composition. Three main groups of river basins were included:

A. river basins disturbed by human activity with a significant influence of runoff from agricultural land, erosion, and drainage systems;

B. river basins with a preserved oligotrophic (nutrient-poor) natural character;

C. river basins where, thanks to the introduction of effective protection, improvement and transfer from Group A to Group B have been achieved.

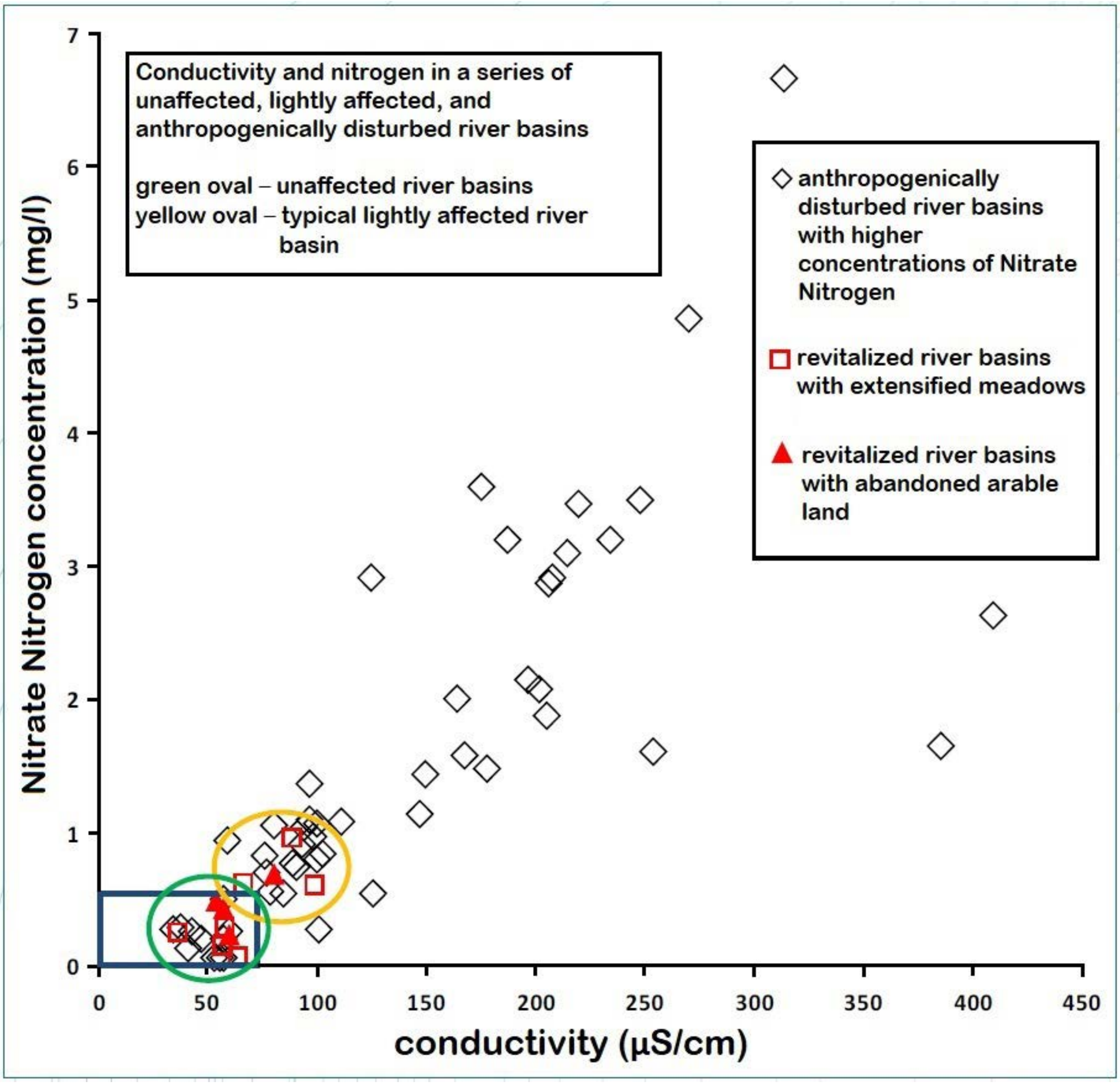


Fig. 227. Large differences in nitrate nitrogen content in water between different types of river basins in terms of land-use, and the requirements of FPM (parts of river basins with FPM occurrence are marked with blue rectangle). A complete list of limiting parameters of water chemical composition is given in Table 4, Annex 1.

A similar dependence is found for calcium; it must be maintained in naturally low values below 8 mg/l in FPM river basins.

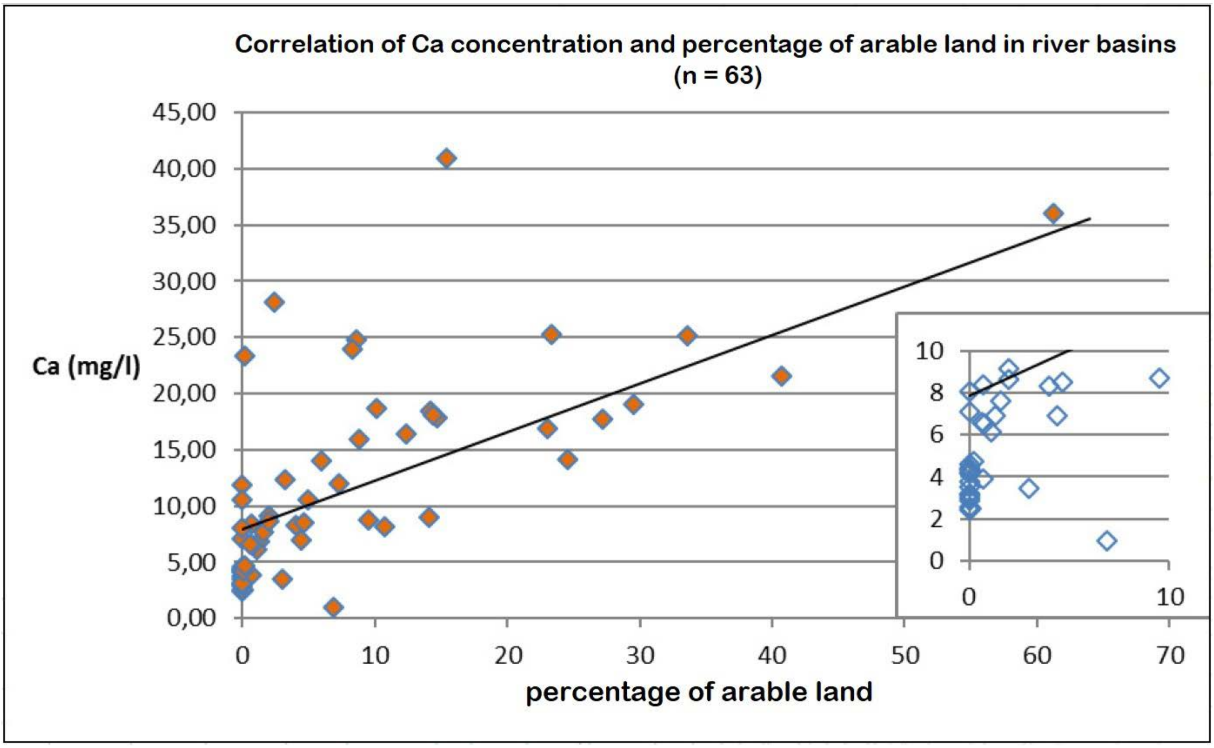


Fig. 228. Correlation between calcium content in water and share of arable land in river basins. Arable land significantly increases leaching of calcium into the water (due to liming, fertilization with organic fertilizers, ploughing, and drainage systems). On the right, there is a detail of a part of the graph with values favourable for FPM (below 8 mg/l Ca). Such low values are only found in river basins with a proportion of arable land below 7 %, but most often 0 % (arable land completely missing). Conversion of arable land into extensive meadows led to the improvement of water chemical composition in a number of small-scale protected areas.

Annex 4. Functioning of RRFs in the 20 years since their construction, focusing on the oldest (LRRF)

Rearing and reproduction features are the essential components of the Czech Action Plan infrastructure (although we primarily always strive for the renewal of the entire river basin). In the long run, their development, construction, and maintenance require a lot of effort and attention. This Annex aims to outline what has been achieved in this area and, in particular, to point out the difficulties and issues that may move our knowledge forward. The text points out only some aspects of the issue and, due to the limited scope and availability of the necessary data, does not claim to be complete.

The following evaluation concerns three RRFs: LRRF – on Lužní potok stream, SRRF – on Spálenecký potok stream, and ZRRF – on Zlatý potok stream.

RRF Lužní potok (LRRF, Aš district, Saale River basin)

LRRF was created between 1996–2001, with the final release of juvenile pearl mussels from the local population in June 2005 (848 specimens aged six and 4 aged four years).

The gravel bed technical design and the riverbed shaping was carried out according to the project's requirements; however, the required quantity and quality of the covering soil was not delivered. In addition, the covering soil was heavily over-calcified due to a major technological error. This was due to the fact that the original strongly acidic and iron-encrusted soil, including turf, was neutralized with 9.8 tons of limestone. Furthermore, 30 tons of clay and 100 tons of straw were added to it, everything was composted and, according to the project, the final product was to be mixed with imported quality soil in a ratio according to a soil composition of about 1:5. However, the contractor did not manage to obtain the required soil, so only the starting, predominantly organic substrate was used. This caused hyporeal toxicity with up to 100% juvenile FPM mortality in bioindications within the hyporeal (conductivity exceeded 200 $\mu S.cm^{-1}$ at multiple sites, ammonium ions up to 1.0 mg/l in one probe–) and the impossibility of maintaining the release of juvenile stages from rescue rearing in the RRF environment. Individuals migrated intensively downstream to the main flow.

After a few years, only the control sample remained in the cage in the RRF, which for many years did not show the necessary growth of the ligament (which connects the pearl mussels' shells) compared to the progressive corrosion (**Fig. 229**). In addition, average growth did not exceed 4%, but survival was almost 100%. Only a few free juveniles were observed.

It was only after 2008 that the corroded proportion of the shells of these FPM began to shrink, together with a slight acceleration of growth to a level of around 8% (**Fig. 229** and **Fig. 230**). In comparative measurements in 2001, the growth of juveniles of the 7+ cohort in the cage was slightly faster than in the Lužní potok stream at the LRRF, but about five times slower than at SRRF in Blanice River floodplain (Bílý et al. 2008).

The remaining individuals from the cage were freely released into LRRF in 2014 at the age of twenty, at an average size of about 4 cm (**Fig. 231**, **232**). In warmer and better food-supplied conditions, the control individuals in Blanice River reached the size of 4 cm at ten years of age. Released individuals were marked with plastic tags. In 2011, the numbers A037–A041 were affixed to them. An inspection took place in 2014 – individual A041's tag fell off; this individual and the others remaining in the cage were marked with the numbers A249–A254. Control individuals in the LRRF cages usually did not bury themselves in the substrate, apparently trying to escape from an unfavourable position.

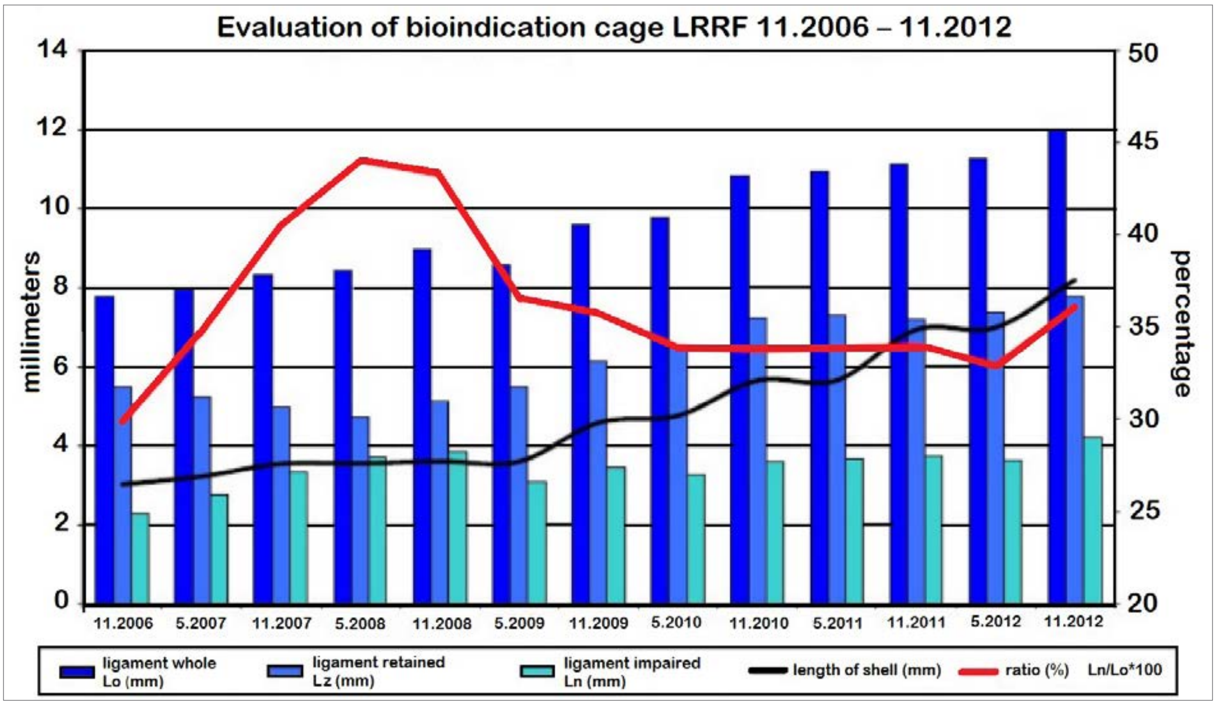


Fig. 229. Evaluation of changes in ligament size and its corroded part and length of juvenile FPM shell (black line) in LRRF bioindication cage, 2006–2012. Red line shows evolution of shell disruption rate. Data: NCA CR and TGM WRI, taken from NCA CR internal material (AOPK ČR 2013b). Graph: O. Spisar.

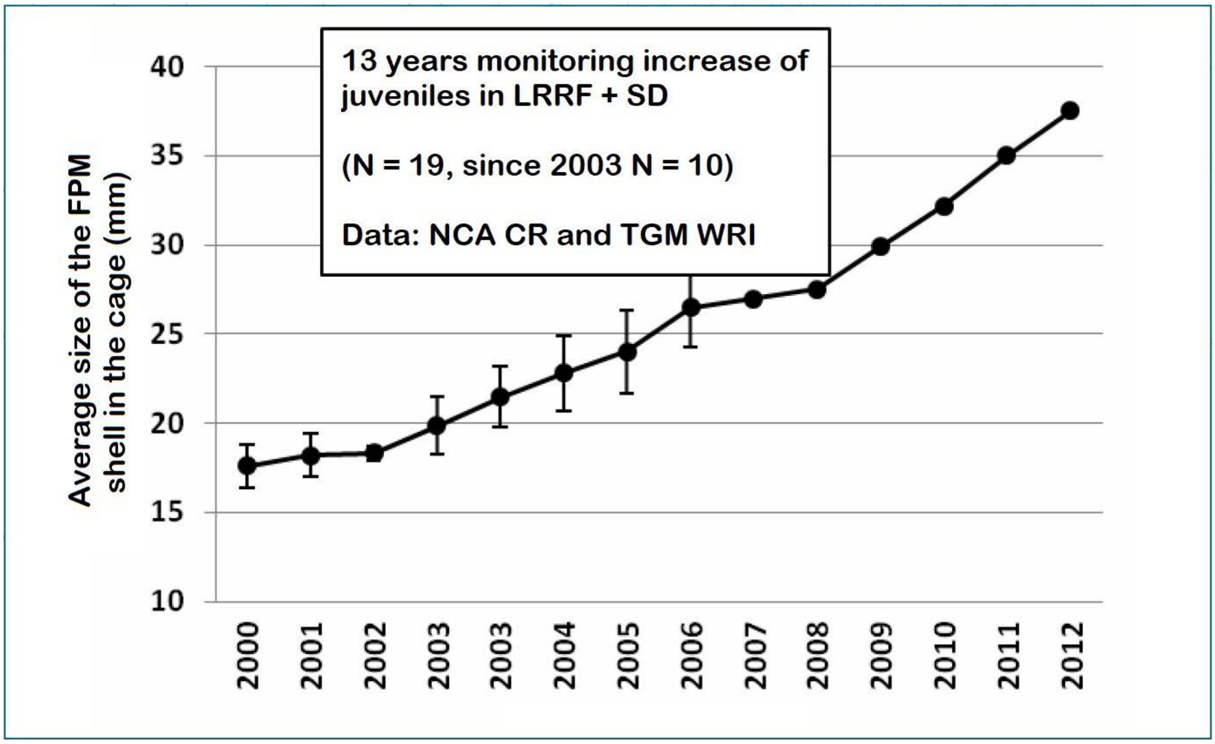


Fig. 230. Development of average shell size (mm) of 19 survivors out of 20 pearl mussels (2000–2003); subsequently (2003–2012) only the 10 largest pearl mussels in LRRF bioindication cage. Measurements always took place at the end of the vegetation season. Measurements taken by J. Hruška (2000–2005) and O. Spisar (since 2006).

Fig. 231. Releasing individuals from FPM control group reared in LRRF bioindication cage, 2000–2014.

Fig. 232. Detail of FPM individual with a mark (number) and a scale. Photo: O. Spisar.

Bioindications performed in 2015, using 1+ individuals in LRRF and in open water at other sites in the vicinity, showed significantly better growth in LRRF compared to the plate installed simultaneously in the stream.

During the gradual leaching of the RRF hyporeal, more favourable conditions were probably established than the ones that prevailed here after the technologically incorrect foundation of the river branch. Unfortunately, in the past fifteen years, the LRRF hyporeal has not been monitored at all. Thus, an opportunity was missed to gain unique knowledge about changes in soil, vegetation, and hyporeal of alluvial meadows on gravel substrates in an environment threatened by acidification. Vegetation development has also not been monitored in more detail; however, there are reports available of the spread of unfavourable herb species, such as rushes. Meadow management has been permanently provided at the RRF since its establishment. Changes in vegetation as well as soil or compost composition deserve a more detailed evaluation. However, for capacity reasons, permanent botanical areas of phytosociological relevés from 2016 (as stated in **Chapter 9**) were only established in the Blanice River basin, where there is hope for their longer-term maintenance. The water chemical composition in the Lužní potok stream, which is the only significant source of water for the RRF (supplemented by one to two cold springs according to the water content of the stream), also has an adverse effect. A detailed analysis of water chemical composition is available on the website www.zachranneprogramy.cz (more recent data) and in the publication of Bílý et al. (2008); older data can then be drawn from the German-Czech brochure (Hannsmann 1996). The documented fluctuations in the concentration of some parameters are certainly unfavourable for FPM. Sudden changes in trophy caused by the construction of a sewer also had an adverse effect on this site.

One of the positive examples of the operation is the use of LRRF for temporary safe placement of adult FPM from Bystřina stream during its repeated drying. The fate of individuals released into Lužní potok stream from stocks in LRRF before 2005 is not exactly known. It is documented that immediately after release, they intensively migrated downstream. No empty shells in larger numbers were found in the river branch, and below the stream up to Fojtské rybníky (two ponds upstream from Huschermühle), subadult individuals of the appropriate age are rarely found in the main flow. In 2016, for example, 7 subadults were found in the section of Lužní potok stream below LRRF, of which one was a marked individual originating from a bioindication cage released in 2014 (O. Spisar, pers. comm.; see **Figures 233** and **234**). It can therefore be assumed that most of the released individuals migrated intensively downstream and at least a small part survived in the main flow of Lužní potok stream. The numbers of individuals from the stocks found directly in LRRF were usually small (after a full-day survey in 2003, J. Hruška states only three individuals buried up to the edge of the mantle). In 2011, O. Spisar found 38 subadult individuals corresponding to the age-appropriate to the released cohort, and in 2016 seven subadults, two of them with the numbers A050 and A051 (O. Spisar, pers. comm., November 2016). However, according to Larsen, 65 % of individuals up to 40 mm in size are completely immersed in the substrate in Norwegian sites (Degerman et al. 2009), and thus LRRF numbers may not be final because occurrence in the substrate makes it impossible to find buried individuals.

A detailed evaluation of the long-term function of LRRF is certainly needed. The comparison of all available parameters for the RRF and Lužní potok stream will be facilitated by a large amount of older data from Lužní potok stream, some of which have also been published (Hannsmann 1996, Bílý et al. 2008). There was long-term bioindication using the cage and currently several comparative bioindications have been performed here (**Fig. 235**). Other extensive data files are stored in the NCA CR archive, which is undergoing digitization for better accessibility. After twenty years, critical evaluation of the RRF construction should be possible.

Fig. 233. Control cage with FPM (LRRF, 2000–2014) in 2014 (size of individuals around 40 mm). Average annual growth for the first 12 years of exposure in LRRF was 1.5 mm (over 2.5 mm was recorded only in 2006, 2011, and 2012). Percentage increase between 2000 and 2003 (around 4 %) gradually increased to 8 % (since 2008). Up to 2003, only one of 20 individuals died, with mortality at zero since 2003. Photo: O. Spisar.

Fig. 234. The same control cage with juvenile FPM (installed at LRRF in 2000) eight years earlier, i.e. in 2006 (average size of individuals only 26 mm so far).

RRF Spálenecký potok (SRRF, Prachatice district, Blanice River basin)

The SRRF was created in 2000–2005, with the release of adult juvenile pearl mussels from rescue rearing and a colony of adult pearl mussels of the local population.

The technical design of the gravel bed, the formation of the riverbed, the quality and quantity of the cover soil and vegetation elements according to the project requirements were in order; however, the required condition was not achieved so that the slope and design of the riverbed prevented sedimentation of fine sediments in the riverbed. Simultaneously, stabilization of the banks and the riverbed of the permanently flowing and eroding forest drainage ditches was to be (but was not) addressed in the catchment area above the RRF. Due to the considerable sedimentation of erosive sediments, juvenile FPM from rescue rearing, as well as adults from the established colony (except for a few specimens), were gradually washed into the main flow. The function of the hyporeal after construction completion was not evaluated and thus no knowledge usable for other constructions of this type was obtained.

Despite annual meadow management with composting of cut biomass with the appropriate addition of $CaCO_3$, there has been a significant decrease in exchangeable soil acidity since completion of construction (when the cover soil had the optimal pH after modifications). According to the evaluation carried out in 2013, the pH/KCl of the soil (exchangeable acidity) decreased to 4.84. It is important to know the causes of this condition due to the adjustment of the method of management for RRF and also for the implementation of meadow management in other sites or the construction of new RRF.

SRRF bioindication assessment was performed repeatedly in 2012–2016; partial results are shown in **Fig. 35**. Comprehensive evaluation of the effects of this RRF would need to be made following new measurements in the LRRF hyporeal.

RRF Zlatý potok (ZRRF, Prachatice district, Blanice River basin)

ZRRF was completed in 2000, with the release of grown juvenile pearl mussels of the local population from rescue rearing and the establishment of a gradually replenished colony of adult pearl mussels from the flooded parts of the main bed of Zlatý potok stream.

The design of this RRF and the supplementary food feature is different. It is not a construction with an artificially created gravel pit and soil treatment with the establishment of a temporarily boarded riverbed. Instead, the manual (spade) method of forming a significantly longer riverbed with a parallel food feature on a smaller tributary was used, while maintaining the integrity of the riparian turf and layering local river gravel on the riverbed in only a few suitable positions, without construction modifications. The cost of setting up this type of RRF was approximately 70,000 CZK, compared to approximately 2–3 million CZK for each of the LRRF and SRRF constructions.

However, the function of ZRRF is relatively good, the flow of water under the rhizosphere for food supply (detritus) is provided by numerous burrows of *Arvicola amphibious* and other soil animals. The development of riparian vegetation is favourable, as shown in **Figure 236**. In the evaluation in 2013, meadow management also showed the expected improvement in the pH of the local soils. The disadvantage is the low water temperature (a phenomenon common to all RRF to varying degrees) which, however, must be addressed in the entire catchment area of Zlatý potok stream above ZRRF.

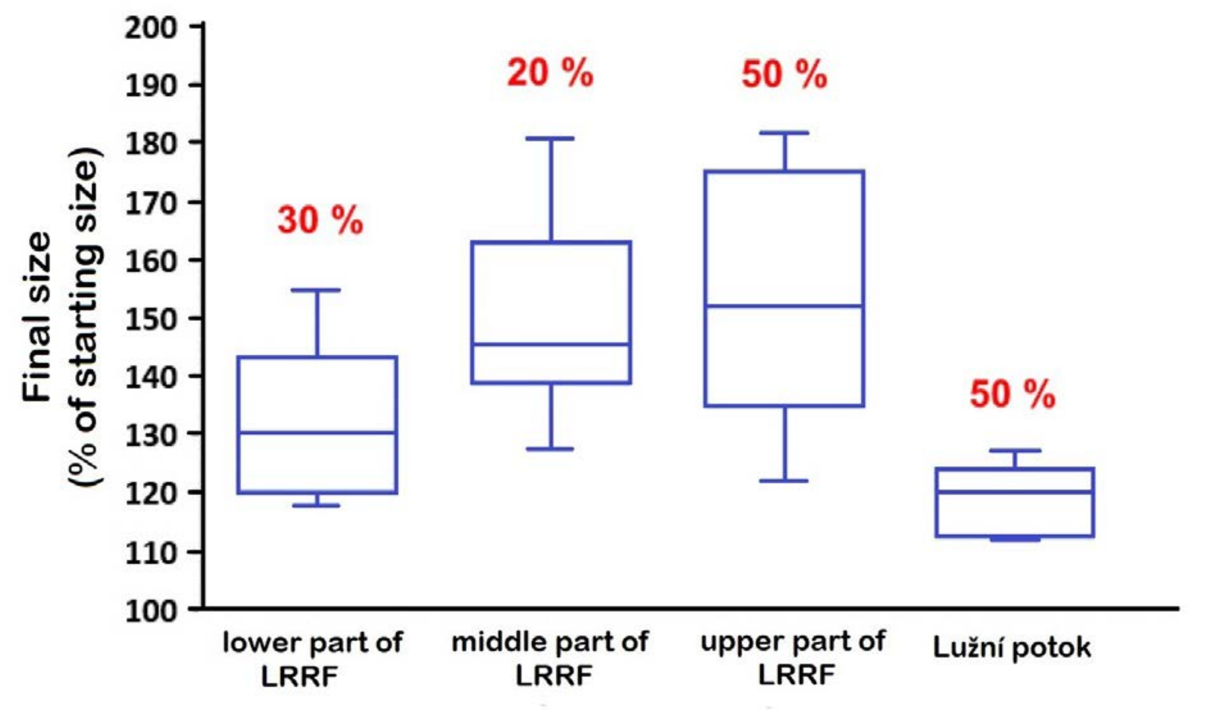


Fig. 235. Three-month bioindication of individuals of 1+ pearl mussels performed by O. Spisar at three places in LRRF and in Lužní potok stream in 2015 (always one plate with 10 individuals at the site); percentage mortality in individual plates marked in red.

Fig. 236. Riparian vegetation on ZRRF with abundant representation of *Chaerophyllum hirsutum*, which mechanically strengthens the banks and produces suitable detritus for FPM.

Annex 5. Composition of detritus in four types of springs and factors characteristic for the helocrene

O. Simon, K. Tichá, K. Douda et al., unpublished data.

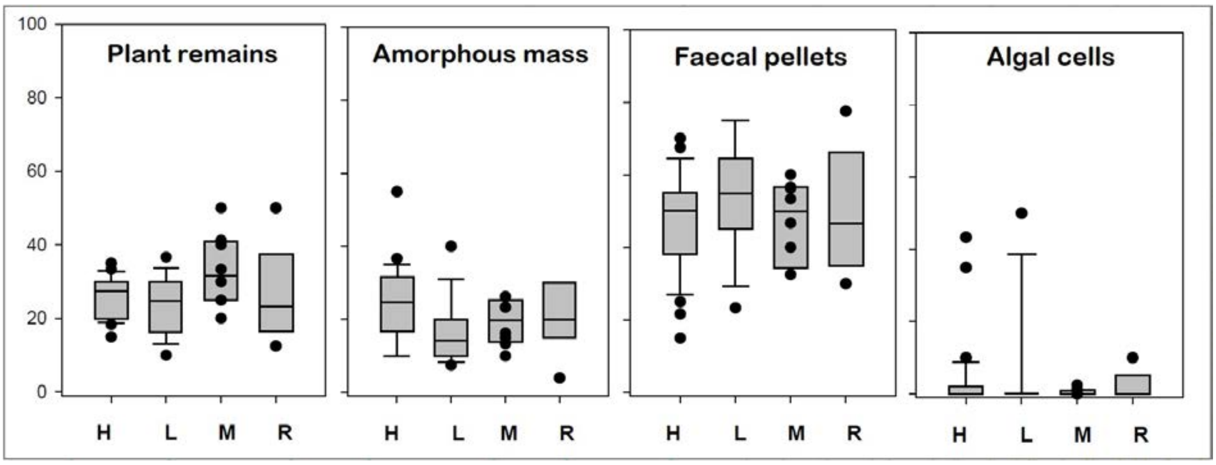


Fig. 237. Composition of detritus (percentage of the field of vision) in four types of springs (microscopic analysis of 83 permanent springs in the Blanice River basin). Helocrene-type springs are used as a source of detritus for rearing. H – helocrene (spring wetland), L – limnocrene (pool with a drain into a stream), M – spring of mixed type, R – rheocrene (stream or direct drain).

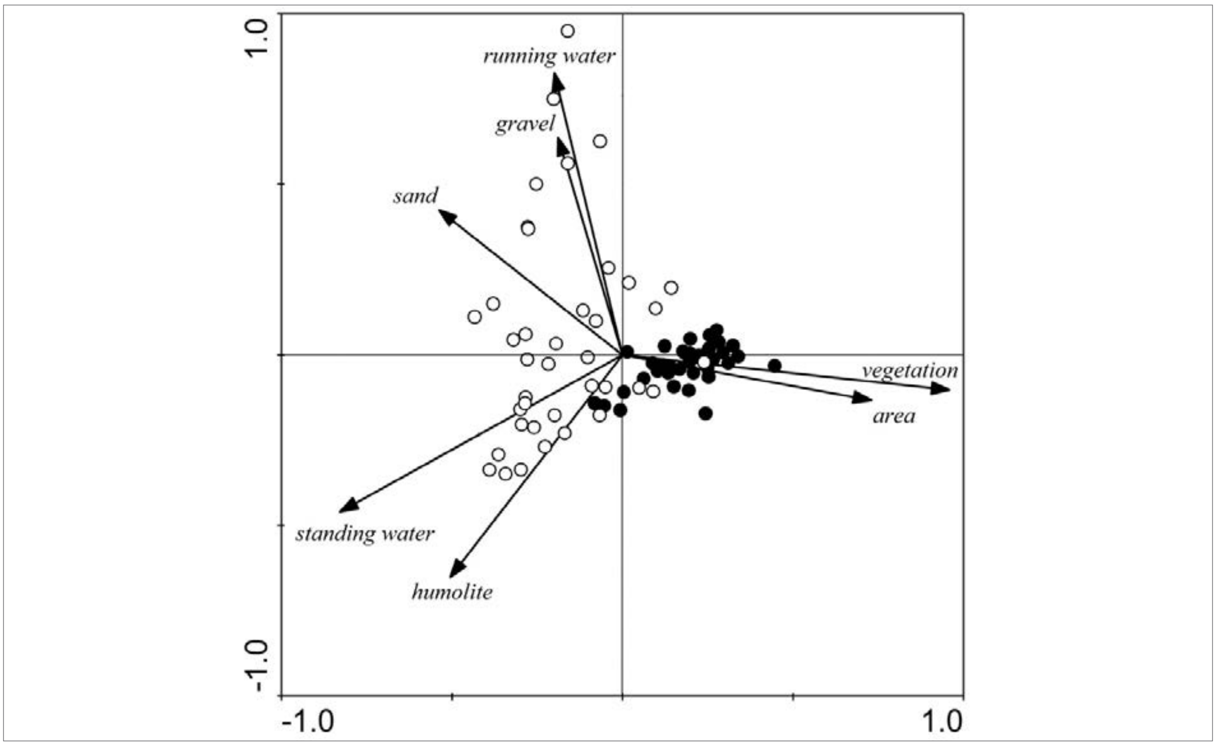


Fig. 238. Characteristic helocrene factors (multivariate PCA for 83 springs in Blanice River basin, explained variability 39 % for the first axis and 28 % for the second axis). Characteristic helocrene (black dots) properties are mainly a large proportion of vegetation in the spring and a larger area of the spring.

Fig. 239. Types of springs. Helocrene.

Fig. 240. Limnocrene.

Fig. 241. Rheocrene.

Annex 6. Calcium metabolism and long-term monitoring of the effect of liming on Boubín mountain 1989–1999

J. Hruška, with modifications by O. Simon.

Juvenile and subadult FPM, which have been growing rapidly, are strongly dependent on detritus calcium content, which was verified by a series of experiments in the 1990s. As described in the summary report from the Action Plan for 1996–1999 (Hruška et al. 2000b), insufficient calcium supply can lead to shrinkage or perforation of shells and death of individuals. Compared to that, dissolved calcium has an adverse effect.

This finding was repeatedly verified from 1993–1998 by field experiments (prior to the design of large RRFs), in which a sedimentation pool was created in front of a rearing channel. The rearing rill, established on an artificially established gravel layer, covered with soil and a suitable composition of vegetation, provides a good food supply, including calcium for the development of shells for young FPM living in the interstitial. These young stages receive detritus from falling root hairs, which arises directly above the gravel layer of this facility. However, it is only transported by the transverse flow between the meanders inside the interstitial and does not enter free-flowing water (**Fig. 242**). Young FPM can grow well in the interstitial. When they reach a size larger than 2 cm, they already begin to filter free water. If the detritus carried by the free water does not have a sufficient calcium content, growth slows down and, in the case of using a preceded sedimentation pool capturing water-borne detritus, growth stops. Simultaneously, their shells slowly reduce in size.

It is not yet known whether this reduction is a direct consequence of corrosion or whether the bivalve is able to use part of the calcium from the peripheral parts to strengthen the shell at the points of imminent perforation. Attempts to place young FPM stages in a section of the stream with low-quality acidic detritus led to complete perforation of shells around the peaks and subsequent death (**Fig. 243**). These processes take place even though the water contains ionic forms of calcium at values around 5 mg/l, which confirms the hypothesis that FPM can only use ionic forms of calcium from free water to a limited extent and their calcium metabolism is largely dependent on calcium contained in falling vegetation. Insufficient calcium supply mainly threatens FPM during the period of intensive growth (young regime) under the age of thirty. Interesting findings were obtained from the evaluation of changes in detritus trophy in the Cikánský potok stream spring area (tributary of the Blanice River) in the Boubín Mountains, where aerial liming of forest stands on the northern slope of the Boubín mountain was carried out in the early 1990s. This was carried out in two waves, in 1990 and 1991, when coarser dolomitic limestone (grain size 0.5 to 6.0 mm) was applied at a rate of 3–5 t/ha. The limed stands were within the sub-basins of Pravětínský potok stream and Cikánský potok stream, where FPM are not present. To obtain knowledge of how a similar intervention would show in the mortality and life processes of this bivalve (FPM are significantly affected by calcium), if it should occur in a river basin with a FPM population, cages with juvenile pearl mussels were installed in the control profiles of the spring area before the liming., while organo-mineral detritus was taken from these profiles and laboratory bioindications were performed (growth and mortality were assessed at five-day intervals).

The results showed that the liming did not increase mortality in comparison with the control station in Blanice NNM. In the control cages, located in tributaries at an altitude of 950 to 1000 m, there were no growth changes of individuals due to low water temperature – the effect of water temperature on the growth of FPM is reported by Hruška (1992a). However, a significant difference showed in laboratory testing of the detritus, when at a temperature above 15 °C the degree of decomposition of organic components began to increase and at this stage juvenile FPM began to grow significantly in shells and body mass. Comparative samples of detritus from NNM Blanice, which contained less degradable organic material, only allowed very little growth.

The measure of the food quality for these filterers has thus proved to be the ability to decompose the organic components of detritus, which, however, must not exceed the limit of possible toxicity and calcium content in detritus. **Table 5** shows the results of the Cikánský potok stream detritus analyses at the time of the best growth of juvenile FPM in comparison to Blanice detritus (also see **Figure 26**, which compares calcium concentrations in differently sized detritus flakes from Blanice River basin springs). The average growth of the shell length of juvenile pearl mussel of the first growth period (0.5 to 1 mm) in five-day bioindication experiments in the laboratory environment are shown in **Figure 244**. The maximum growth was reached 2 to 3 years after forest liming, i.e. at a time when the supplied calcium and magnesium were already sufficiently contained in the dying organs of vegetation. In the following years, growth of juvenile FPM gradually decreased again. However, the findings must not lead to the conclusion that blanket forest liming is a suitable measure to improve food conditions in oligotrophic river basins with FPM. This intervention significantly accelerates the mineralization of humus, and thus the mineralization of spring waters. Temporarily improved trophy of organic detritus for FPM young stages is only a short-time phenomenon; this is because the rapid degradation of organic matter reserves by applying limestone to the surface soil layer leads to further reduction of ecosystem stability after leaching of mineral components.

However, findings from the development of detritus trophy in the Boubín Mountains have allowed understanding of the causes of FPM stagnant reproduction and development of food management methods in which calcium is applied to compost and only the acquired humus-forming material is returned to riparian land in suitable locations of FPM watercourses. However, the food of young pearl mussels is not this humus-forming material, but a fresh fall of plant matter, subsequently grown on improved soil.

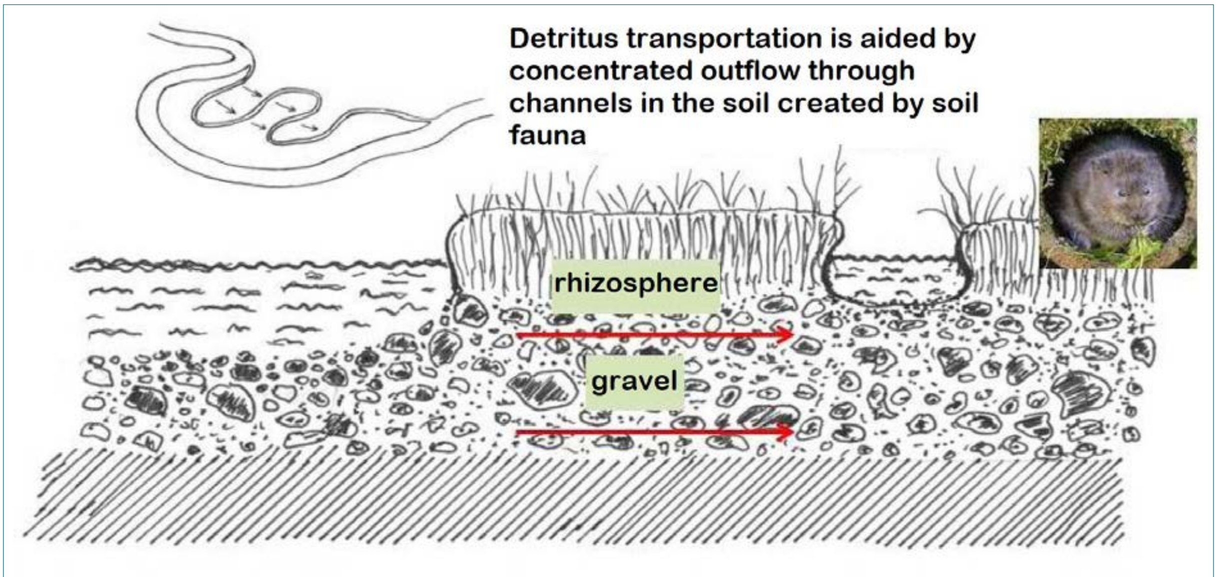


Fig. 242. Subsurface flow of water through the gravel layer between RRF meanders or crevices in natural gravel floodplain. The products of the falling root hairs are introduced directly into the interstitial spaces in the hyporeal (into the aquiferous environment below the bottom of the stream). Drawing: J. Hruška.

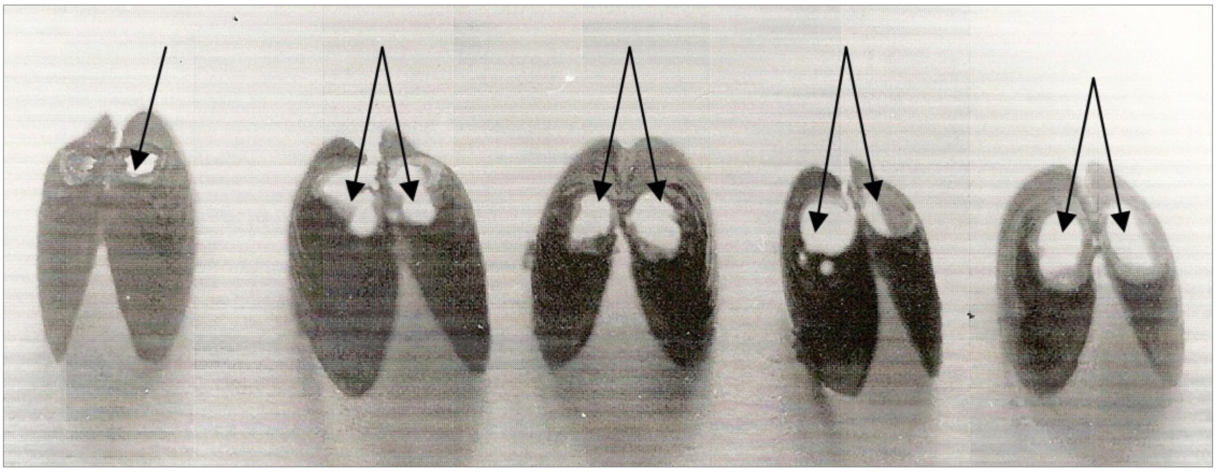


Fig. 243. Complete perforation of young pearl mussel shells (5 to 7 mm) in habitats with insufficient food quality in a cold and precipitation-rich year (Hruška 1999).

Table 5. Chemical composition of Cikánský potok stream detritus at the time of best juvenile FPM growth in comparison with Blanice detritus. pH/H2O – water reaction, pH/KCl – exchangeable soil acidity.

	Detritus from Blanice River (site 14)	Detritus from Cikánský potok stream (site 3)
pH/H2O	5.4	5.4
pH/KCl	5.1	4.9
Ca (mg/kg)	2760	4250
K (mg/kg)	260	209
Mg (mg/kg)	428	654
P (mg/kg)	10	30

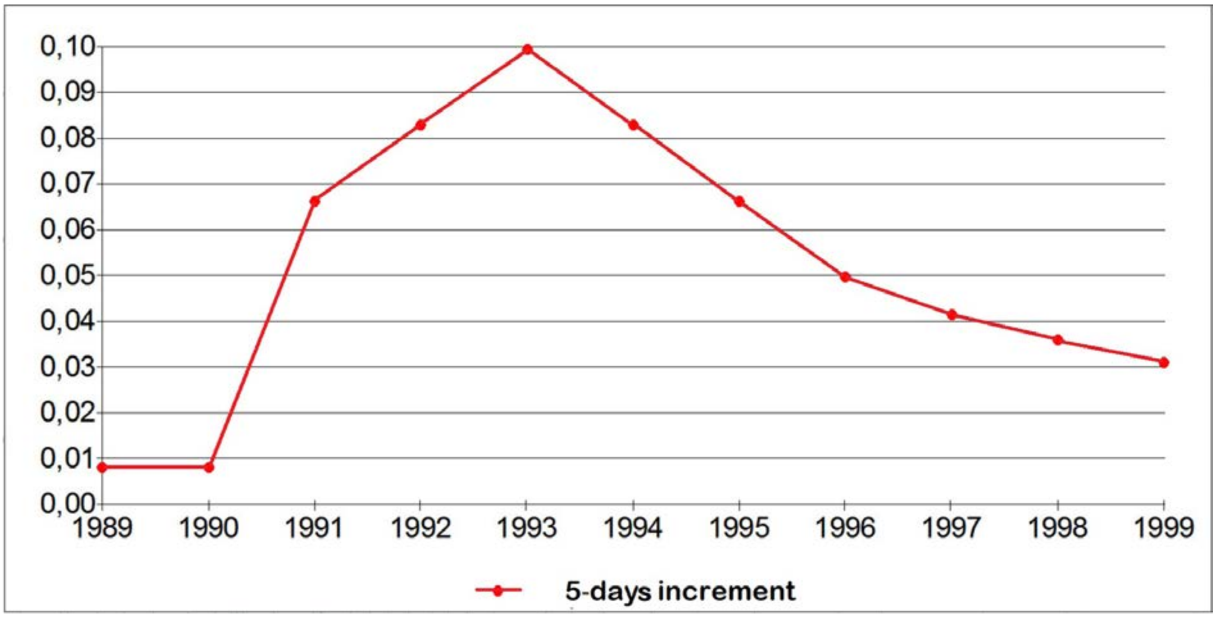
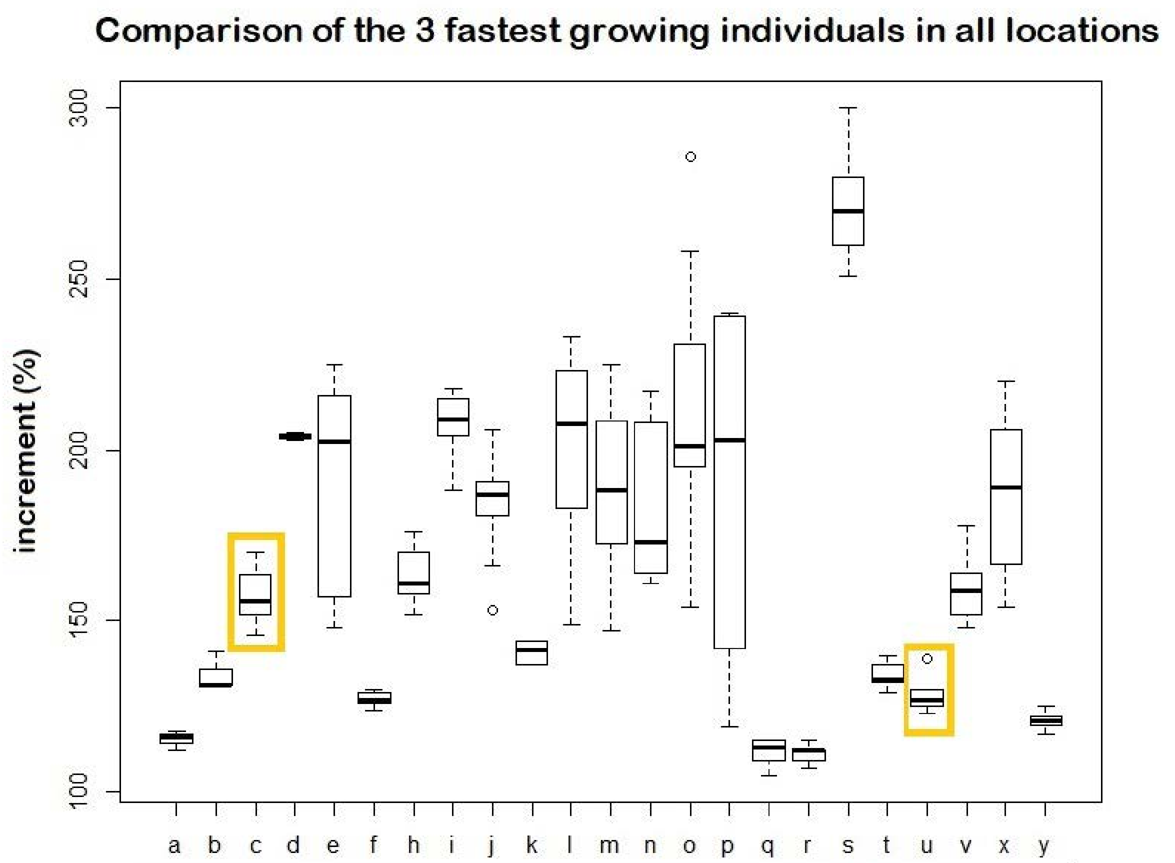


Fig. 244. Average five-day growth in 1+ juvenile pearl mussels in mm (N = 10) during laboratory evaluation of detritus samples from Cikánský potok stream basin affected by aerial liming in 1990 and 1991 (see Chapter 7.3.1 for more information about the method). Liming did not change the mortality of individuals kept in cages directly on site.

Annex 7. Addition of 1+ pearl mussels in sites in the Blanice River and Zlatý potok stream basins in 2015

The summary graph (**Fig. 245**) clearly shows four groups of sites: profiles with only minimal growth of juveniles (a, q, r), then a group of tributaries with growth up to 150 % of the input size (b, f, k, t, u, y), and three sites with the FPM occurrence, where there was a balanced growth around 160 % (c, h, v). The remaining sites with growth reaching values of about 200 % and with usually a large dispersion of growth lie on the main stream the Blanice River. Zbytinský potok stream (s) stands out with exceptionally fast and in 2015 balanced growth. Blanice River below Zbytinský potok stream (p) then shows the largest variation of growth from 140 % to 240 %.



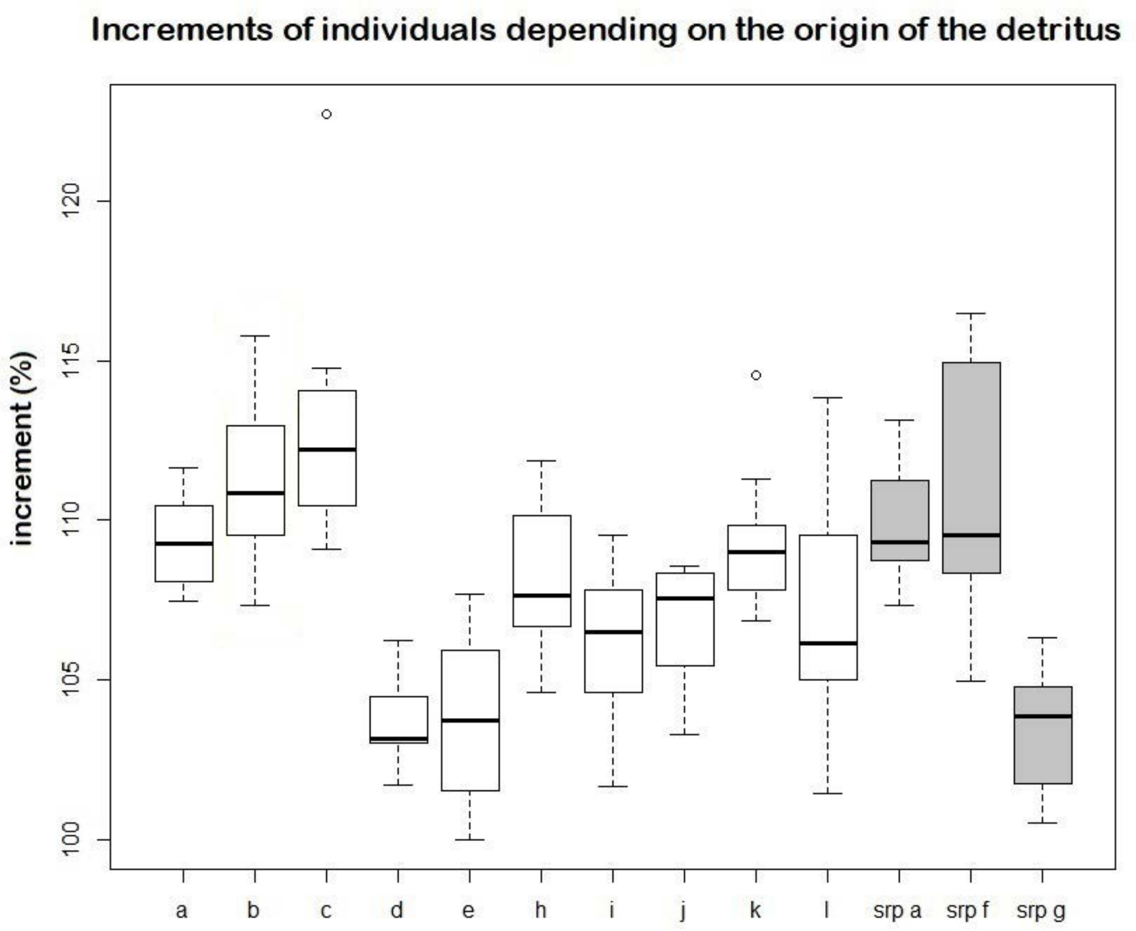
a	Hornosněženský potok str. - mouth	**m**	Blanice R. below Spálenec RRF centre
b	Spálenecký potok str. - above revitalizations	**n**	Spálenec RRF centre above main colony
c	SRRF - mouth	**o**	Blanice R. above Zbytinský potok str.
d	Blanice R. above SRRF	**p**	Blanice R. below Zbytinský potok str.
e	Blanice R. below SRRF	**q**	Luční potok above Čapí mokřiny marsh
f	Tetřívčí potok str. – bridge	**r**	Luční potok below forestry spills
g	Tetřívčí potok str. – tributary from Vyšné	**s**	Zbytinský potok str. - moth
h	Tetřívčí potok str. – mouth	**t**	Zlatý potok str. above ZRRF
i	Blanice R. above the Sněžný potok str.	**u**	ZRRF
j	Blanice R. below the Sněžný potok str.	**v**	Zlatý potok str. - end of NNM
k	Sněžný potok str. - mouth	**x**	Blanice R. at Božejovice
l	Blanice R. above Spálenec RRF centre	**y**	Zlatý potok str. below Skříněřov

Fig. 245. Percentage growth of 1+ FPM at all sites monitored in 2015 in Blanice River and Zlatý potok stream basins. Bioindication method – three fastest growing individuals from each plate. Site g is excluded from the evaluation as it dried up during the tests. RRF values are marked in yellow.

Annex 8. The growth of 1+ pearl mussels in detritus test from Blanice river basin sites in 2015

As can be seen in **Figure 246**, the largest growth was recorded in detritus from the control profile Lapačka (on Spálenecký potok stream above SRRF, c) used in juvenile rearing. The detritus from SRRF (b; marked in yellow) was second best. On the other hand, low values of growth were found in detritus from the Blanice main stream (d, e), as well as from the August 2015 sampling in the feeding area on Sněžný potok stream (aug g). For all values, a large variability of results can be seen. The most fluctuating results were found for detritus from eutrophic inflow from the village of Křišťanov (l) and from the feeding area of the Blanice River taken in August (aug f).

Here it is necessary to point out the data in **Figure 246**, where the percentage growth of 1+ individuals was evaluated in a laboratory test of detritus originating from a feeding area on Zlatý potok stream before its technical treatment (a) and after its treatment in August of the same year (aug a). This modification of a feeding area with a periodic overflow regime was carried out at the end of April 2015 and it will take at least a year for vegetation changes to take effect due to the changed water regime. Not only will the species composition of plants change, but also the representation of food-important components in the aboveground part and rhizosphere. The fall from this vegetation will create a different food supply. The assessment of the impact of April adjustments in August of the same year is therefore very premature; however, it is important as a comparative value.



a	feeding feature Zlatý potok str. before tech. treatment	**aug g**	feeding feature Sněžný potok str. (AUGUST)
aug a	feeding feature Zlatý potok str. after tech. treatment (AUGUST)	**h**	Tetřívčí potok str.
b	feading feature SRRF	**i**	Tetřívčí potok str. - mouth of Vyšenský potok str.
c	control profile Lapačka	**j**	Tetřívčí potok str. - bridge
d	feading feature 16 A - side arm of Blanice R.	**k**	Tetřívčí potok str. - mouth of Bažinný potok str.
e	feading feature 16 B - side arm of Blanice R.	**l**	Tetřívčí potok str. - mouth from Křišťanov
aug f	special feeding feature Boční rameno Blanice (AUGUST)		

Fig. 246. Percentage growth of individuals of 1+ FPM in the laboratory test of detritus (constant temperature of 19 °C) from all monitored Blanice River basin sites. Detritus sampling took place in June and August 2015.

Annex 9. Excerpt from the publication Food requirements of FPM and their semi-natural rearing in the Czech Republic (Hruška 1999) focused on the original description of rearing methods

Excerpt from Hruška 1999. New photographs and a table by J. Hruška are added to the text.

Introduction – Semi-natural rearing of young pearl mussels

The previous several years of conservation management (e.g. treatment of rills or side basins, support of suitable vegetation on a porous base) have created improved food conditions that allow semi-natural rearing of young pearl mussels directly on natural food sources. It is implemented in two basic ways, either freely in the interstitial of the rearing sections of the stream, or in gravel cages located in rearing sections of the stream.

The great advantage of this rearing method is preservation of the predominant share of natural influences (changes in food composition during the season, preservation of natural temperature cycles and natural ways of obtaining food – see **Figure 247**, the possibility of creating natural immune mechanisms, etc.), even in the case of cage rearing. After six years of practical application, it is already clear that the chosen procedure is optimal for the Action Plan.

Semi-natural rearing to preserve the gene pool or improve the age structure of the FPM population for individual sites will continue to be carried out only in the controlled protected area on the Blanice River. Simultaneously, the original sites are being revitalized, including modifications to improve food conditions so that the reared stages can be returned to them after 3–5 years of semi-natural rearing. These are mainly special modifications (side channels or tributaries with an established infiltration layer and conservation management of riparian vegetation, so-called RRF). This environment will provide young FPM with a refugium until the function of springs, the capillary network in the entire river basin, and the function of the soil and its vegetation cover can be restored. Then they will be able to complete their development freely at the bottom of streams and rivers.

Semi-natural rearing procedure

Semi-natural rearing consists of the following sub-procedures, which are described in detail below:

A. Controlled invasion of host fish by larvae of native FPM population

B. Controlled invasion of fish and controlled course of parasitic development of larvae from non-native FPM population

C. Free rearing of juvenile native FPM population during the first growth period

D. Rearing of young FPM obtained by the method of controlled parasitic development during the first growth period

E. Rearing in the second and further growth periods

A. Controlled invasion of host fish by larvae from native FPM population

Only natural reproduction of fish is allowed in the protected area. It is therefore not possible to release young fish from artificial rearing, which would have been previously invaded by pearl mussel glochidia.

Salmo trutta m. *fario*, caught in the upper part of the protected area, are therefore used for a controlled invasion. Fish of all sizes are invaded by pearl mussel glochidia from the local population by bathing in tanks with water containing FPM glochidia, until optimal use of the gill area is achieved (2000 to 5000 glochidia, depending on size). The fish are then released freely back into the stream.

The following year, at a time when the average water temperature is starting to approach 15 °C, the fish are caught again. These are then sorted into two groups. The first group is fish that have gill tissue inhabited by glochidia of pearl mussels from the previous year. These fish remain in the rearing facility until the juveniles are released. Then they are returned to the lower section of the protected area.

The second group is fish that are not invaded by glochidia. These will be temporarily kept in the rearing facility until the glochidia mature so that they can be invaded and returned to the stream. Despite the proven ability of fish to acquire immunity against FPM glochidia (Bauer 1987) and the annual repetition of the procedure since 1984, FPM glochidia persist in fish in sufficient numbers until metamorphosis. Frequently, trout of 18 to 24 cm in size are good hosts as well.

The release of young pearl mussels from larger fish is delayed by 15 to 30 days compared to smaller fish. However, the released FPM tend to have higher vitality and usually a larger initial size of 0.4 to

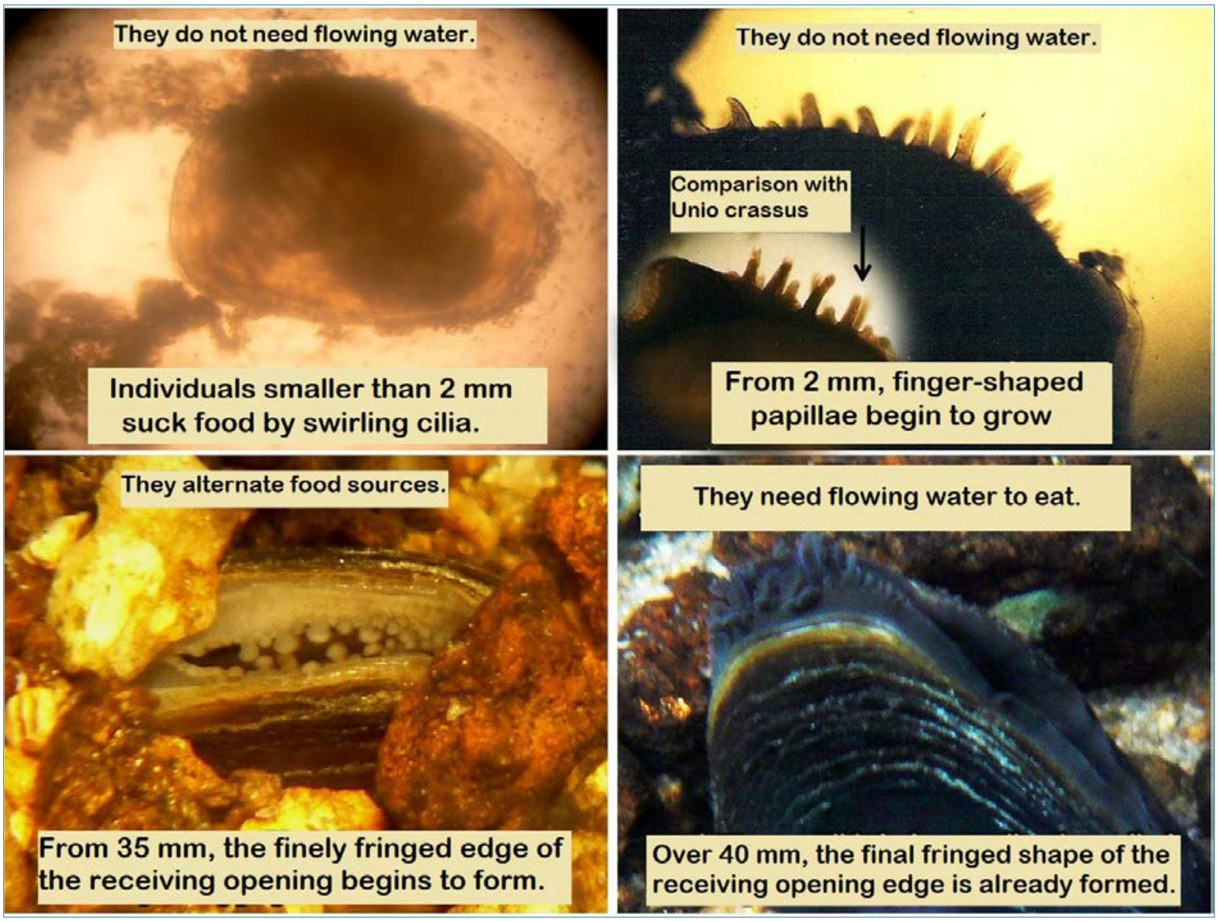


Fig. 247. Food intake method and filtration apparatus development during a pearl mussel's life. Rearing practices must respect changes in the way FPM intake food at different ages.

0.5 mm, compared to 0.3 to 0.4 mm of the initial size of juvenile FPM released earlier from smaller fish. Every year, about 20 % of the invaded fish are recovered. Other fish migrate in the basin and release young FPM into the natural environment.

However, host fish catching, controlled invasion, and redistribution are significant stress factors and interference with natural processes of the protected ecosystem, and therefore their extent is reduced every year. As a substitute, vegetated overhanging banks are established below the pearl mussel colonies, which serve as shelters for fish. Eddy currents carry the FPM glochidia in large numbers into these shelters and, as a result, a high level of natural invasion is achieved here. The fish shelters are then either directly followed by the rearing sections of the stream, where the released young FPM are carried spontaneously, or it is possible to catch a certain number of fish in the shelters before the release of the young FPM. These are then temporarily placed in cages in the rearing section, or in a device from which the released young FPM can be taken into sand cages.

Thus, semi-natural rearing is gradually getting closer and closer to natural conditions. The predation pressure from *Lutra lutra* leads to a constant rapid change of fish stocks and thus reduces the effect of the possible acquisition of fish immunity to pearl mussel larvae.

B. Controlled invasion of fish and controlled course of parasitic development of larvae from non-native FPM population

In the case of controlled invasion of host fish by pearl mussel glochidia from different populations, a completely different procedure is chosen. In this case, fish from artificial rearing can also be used.

Trout with a size of 8 to 12 cm are most often used, already having a sufficient area of gills and at the same time they can be more easily reared in captivity. Fish are invaded by bathing in water with FPM glochidia. They are then kept in cages in flowing water for at least one month, when the parasitic phase of the young FPM development cannot yet be accelerated by increased temperature. It is optimal to keep these fish in this way until the average water temperature drops to 5 °C.

The fish are then transported to the aquariums in a rearing facility, where a gradual increase in water temperature up to 16.5 °C induces a controlled course of metamorphosis and subsequent release of juvenile pearl mussels to the bottom of the aquariums after reaching the required sum of temperatures. Here, the young pearl mussels gather under a protective perforated cover and are continuously removed for the next rearing phase. The fish are returned to their original site after the release of the juvenile pearl mussels and the gradual re-habituation to the ambient temperature.

C. Free rearing of native young FPM during the first growth period

Young pearl mussels freely released from the host fish into the rearing sections of the stream continue to develop quite naturally. The rearing sections only provide them with optimal flow and food conditions. Food conditions are optimized by conservation management of riparian land.

For comparative evaluation and, simultaneously, as a safety reserve in case of a possible accident on the stream, part of the reared population is kept in sand cages located in the rearing sections of the stream. Here, young FPM have very close to natural conditions for their development. Water carrying natural food flows through the cages, the natural dynamics of physical and chemical parameters of the aquatic environment is preserved and, in a limited space, young pearl mussels can move freely in the gravel layer or move to its surface. The mesh of the netting, which forms the walls of the cages for the first growth period, is 0.25 mm; the filling consists of separated sand with a grain size of 1–2 mm. In this type of cage, young pearl mussels are reared until the end of the third growth period, when their size exceeds the size of the grains of the sand filling and they can be easily separated by sieve.

D. Rearing of young FPM which were obtained by the method of controlled parasitic development during the first growth period

The rearing of young pearl mussels which were obtained in aquariums by means of temperature-controlled parasitic development (e.g. breeding for other sites), is different. The time of their release from the fish falls within late autumn and winter. Exceptionally, it is therefore necessary to use temporary rearing in an artificial environment.

Until the formation of the mantle filtration apparatus, the FPM youngest stages live exclusively in the interstitial spaces of the riverbed with very slow water flow and obtain food by active suction or sediment swirling. For this relatively short period (2 to 3 months) it proved to be the most suitable comparative experiment to use, rearing in tanks with stagnant water which is changed at 5-day intervals together with food.

The food consists of organo-mineral detritus, produced by the rhizosphere in semi-natural rearing facilities. It is therefore the same type of food that is used in the wild. The temperature is maintained between 16 and 18 °C. Before use, the detritus is filtered through a 0.04 mm sieve and is dosed in an amount which, after sedimentation, forms a layer about 2 mm thick at the bottom of the tank. During the periodic changing of water and food, the reared pearl mussels are separated by sieve and subjected to control. Mortality, growth of shells, and overall vitality are determined, while dead, sick, and suspicious individuals are separated from rearing. Growth in artificial rearing on natural food has the same growth curve as semi-natural rearing.

After reaching a relative growth of about 250 % in the group of the most vital individuals, the separation of the weakest non-growing individuals is performed by sieves (they fall through a 0.35 mm sieve) and healthy individuals (they are retained by a 0.5 mm sieve), possibly even very healthy individuals (they are retained by a 0.65 mm sieve). Young pearl mussels sorted in this way are placed in rearing sand cages. Containers with cages need to be gradually cooled to free flow temperature. The cages are then placed in outdoor semi-natural rearing systems. This is done immediately after reaching the required 250 % of relative growth in vital individuals (from 0.4 mm to 1 mm), regardless of the size of the less healthy individuals.

After this intensive growth, the rest phase begins, similar to the natural environment. If the cages are placed outdoors during the winter, the rest phase is shorter than in the natural cycle. However, current knowledge shows that it is sufficient. After the watercourse temperature naturally rises above 10 °C in May, the second growth period of this method of rearing begins. After its completion, a natural rest and growth cycle begins.

The alternation of growth and rest periods must be maintained. With the prolongation of the rearing period in artificial conditions, the mortality of less healthy individuals quickly begins. The strongest individuals often grow further; however, delays of various lengths begin to appear during their growth, which subsequently lead to exhaustion and often to death.

When rearing juvenile pearl mussels in tanks with detritus, it is appropriate to rear them together with a small amount of *Peracantha truncata* water fleas (formely *Pleuroxus truncata*). These water fleas clean the surface of the young FPM shells and remove remnants of mucus and faecal pellets from them. Thus, they provide good natural sanitation.

E. Rearing in the second and further growth periods

In semi-natural rearing facilities, the development of freely released individuals only takes place with the control of flow rates and conservation management for the good function of the riparian land rhizosphere.

In individuals kept in sand cages, it is necessary to check the flow rate of nets, depending on the situation. The cages, which are immersed in the riverbed flow profile, are almost maintenance-free.

However, they are only suitable for individuals with a shell length of less than 2 mm. Larger individuals must be able to filter even free-flowing water. Therefore, after reaching this size, they are moved to new cages with a bigger mesh.

From the third growth period, cover nets with 1.4 mm meshes and a filling with 2–4 mm gravel grains can be used. These cages are attached to the stones in free flow, and the young pearl mussels choose between obtaining food trapped in the sediment or food freely carried by water. **Table 6** shows the scheme of the basic rearing cycle up to 5 years of age. The oldest reared FPM in 1996 are documented in **Figure 248**. The same age group at the time of entry into fertility is documented in **Figure 40**.

Table 6. Semi-natural rearing with temperature-controlled metamorphosis and cage rearing up to the fifth growth period at the expected output of 500 individuals (basic rearing cycle).

Growth period (beginning)	1.	2.	3.	4.	5.	End of 5. g. p.
Starting n. of indiv.	5000	500–2500	500–750	500–600	Approx. 500	
N. of indiv.	500	250	150	100	50	
N. of cages	10	10	5	6	10	
Sand grain size (mm)	Container with water and detritus exchanged every 5 days, without sand	1–2	2–4	2–4	2–4	
Sieve mesh size (μm)		333	700	700	700–1400 (according to the size of indiv.)	
Age (months)	0	2	14	26	38	50
Average shell size (mm)	0.4	1	2.8	5.4	8.9	13.2
Max. shell size (mm)	0.5	1.2	3.8	8.5	12	17.6
Min. shell size (mm)	0.3	0.9	2.1	4	6.7	11.1
Average increment (%)		250	280	193	165	148
Cage filling		Sand with grain size 1–2 mm	At the end of the 2nd growth period, it is possible to separate some of the optimally growing indiv. on a sieve, and leave the others for another year	At the end of the 3rd growth period, separate the rest and put it in a cage with 2–4 mm grain size sand	Sand with 2–4 mm grain remains	

Optimal growth of young pearl mussels

As habitat conditions can significantly affect the growth of young pearl mussels, but also the lifespan and reproductive capacity of the population, optimal growth rate is an often-discussed issue.

The revitalization of selected river basins needs to be directed more towards a habitat that will allow FPM to grow slowly and survive for a long time. However, it is not easy to fulfil this intention, due to the great disturbance of the buffering capabilities and the overall properties of the soils of the source areas of the FPM streams.

The main difference between the habitats of faster-growing short-lived forms and slow-growing long-lived forms is probably this:

Short-lived forms of fast-growing pearl mussels live in waters into which permanently larger amounts of nutritionally rich organic substances enter from the river basin. In the riverbed interstitial, oxygen conditions (decomposition processes and low flow rate) are often subpar for the development of young FPM, so therefore they must stay close to the surface. However, because free-flowing water provides enough nutrients, young FPM can grow well here. However, this short-term advantage is subsequently suppressed by the need to increase their metabolism speed. This significantly reduces the lifespan and reduces the reproductive potential.

Long-lived forms of slow-growing pearl mussels live in streams where free flow provides little nutritious food. On the other hand, the riverbed interstitial can be supplied with sufficiently nutritious food from the rhizosphere of riparian land. Thus, the young FPM stages have the conditions for sufficiently fast growth here, as long as the function of the soil and vegetation is not weakened. During their growth, young FPM can gradually alternate both food sources. In adulthood, they only

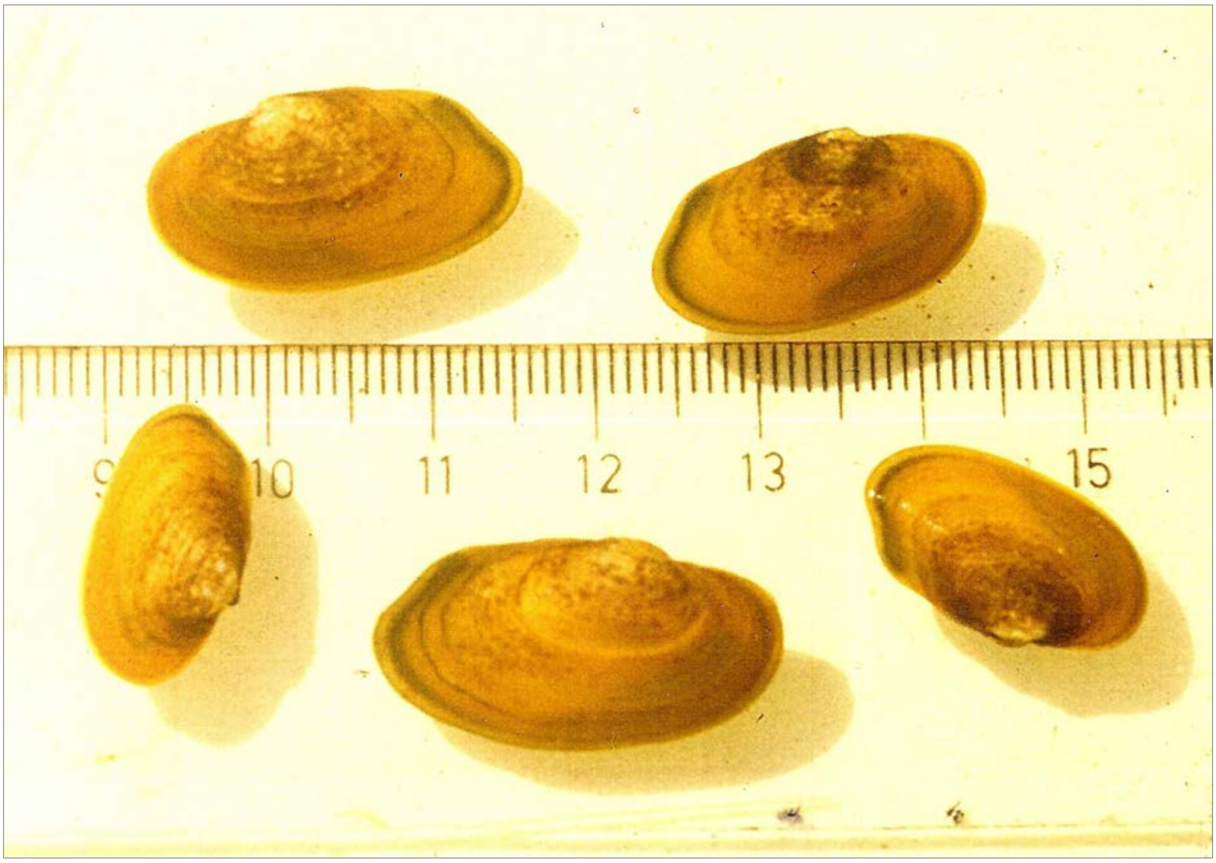


Fig. 248. The largest pearl mussel reared to date, 62 months after leaving the host fish, measured in September 1996. Photo taken from the work of Hruška (1999).

take less nutritious food from free water and their growth rate decreases. In such a case, optimal conditions occur for the growth of the individual and for overall population prosperity.

Without conservation management, however, the habitats of FPM long-lived forms are no longer capable of such a function. The hope lies more in a good balance between the two forms of food supply. Therefore, the Action Plan was focused mainly in this direction and semi-natural pearl mussel rearing is conceived as its part which serves both to preserve the gene pool of individual populations and as a long-term bioindication of changes in the ecosystem during revitalization interventions. However, the basic aim is to revitalize the river basin to such an extent that the restoration of natural reproduction is possible.

Annex 10. Summary of principles for the use of fertilizers and livestock manure on sloping land and close to surface water bodies

Taken and modified from the methodology of Klír & Kozlovská (2012).

On sloping land there is an increased risk of water pollution by soil erosion and surface run-off of applied industrial or livestock fertilizers or excrement of grazed animals and leaching of nitrates by subsurface runoff. The level of nitrogen loss risk depends on the soil and climatic conditions of the habitat, the shape of the land, the length, segmentation and exposure of the slope, cultivated crops, tillage, and the industrial and livestock fertilizers used.

On light sandy soils with good infiltration, leaching generally predominates; in contrast, on heavy clay soils there is a higher risk of surface runoff. When using industrial and livestock fertilizers on agricultural land, it is necessary to prevent their direct penetration into surface waters or subsequent washing away by surface runoff. The requirement for their timely introduction into the soil is important. Neither mineral fertilizers nor organic substances contained in, for example, liquid manure, slurry, and silage effluent must enter the water uncontrollably. During their decomposition, oxygen is removed from the water, which aquatic animals then lack. Another danger is harmful microorganisms and parasites from livestock faeces. Ammonia nitrogen and some other substances contained in farmyard manure are also harmful.

Table 7. Summary of principles for fertilizer and manure use on sloping land. Nitrogen fertilizers are mineral nitrogen fertilizers, organic, organo-mineral, and livestock fertilizers. Recommendations are based on requirements for management in vulnerable areas, according to Government Decree No. 262/2012 Coll.; their observance is mandatory in vulnerable areas (in the form of an action programme) and recommended outside vulnerable areas. Further information on inclusion in vulnerable areas and farming conditions can be found on the Farmer's Portal (www.eagri.cz).

Land	Sloping	Protective belt	Measure
Near surface waters*		At least 1 m	preserve original vegetation
Near surface waters	over 7°	At least 25 m	do not fertilize with manure
Arable land without vegetation	over 3°		immediately incorporate nitrogen fertilizers into soil (within 24 h)
Arable land without vegetation	over 12°		without application of nitrogen fertilizers (except manure and compost)
Permanent grassland	over 7°		limit the one-time dose to 80 kg of total N per ha
Pasture**	over 17°		it is necessary to exclude cattle grazing

* When using fertilizers and livestock manure on agricultural land, it is necessary to prevent their direct penetration into surface waters, or subsequent washing of the fertilizer and livestock manure by surface runoff. Due to increased water protection, the original vegetation must be preserved in a width of at least 1 m from the shoreline.

** There must be no irreversible damage to the turf and muddying of the surface on the pastures, even in the case of year-round residence of animals.

Annex 11. Systematic gradation of land management zones in the vicinity of watercourses

It is necessary to strive for the appropriate use of non-forest areas which complement water management, construction, and other measures and which simultaneously serve water protection, nature conservation, and species diversity – that is, differently according to the distance from springs, wetlands, and streams.

To ensure appropriate use, agreements with landowners with the possibility of financial support should be preferred.

An example of the application of this principle of differentiated management is Horní Malše NM.

Zone I – in the SPA it is defined as all areas of all land; in the protection zone of the SPA, it is located on both sides of the banks of streams, springs and wetlands, on both sides at least 10 m wide; it is a separation and buffer zone at the springs and along the streams.

Land in zone I

- not to be used for economic purposes, or only to carry out management to support the function of habitats,
- remove unwanted vegetation and possibly undesirable structural modifications,
- gradually bring the whole zone closer to the natural state of the environment,
- if necessary, stop the succession of woody plants so that there is no excessive shading of streams.

Zone II – is located in the protection zone of the SPA, starts at the nearest 10 m, and extends at least 50 m from the banks of streams, springs, and wetlands.

Land in zone II

- only extensive use of areas in the entire floodplain or spring area is possible,
- no tillage – only permanent meadows without grazing,
- no use of biocides,
- no fertilization with mineral fertilizers or rapidly decomposing organic fertilizers, slurry, liquid manure, faeces, sludge, fugate, industrial composts, etc.
- fertilization is possible only with quality grass or manure compost from local production.

Zone III – is located in the protection zone of the SPA, starts at the nearest 50 m, and extends at least 100 m from streams, springs, and wetlands.

For land drained by systematic drainage, the drainage system is considered to be a watercourse **in the parts of the river basin up to the first pour point**:

- extensive land use in thalwegs of watercourses,
- no use of biocides,
- no tillage,
- only permanent meadows with the possibility of grazing by cattle and sheep (max. 0.35 LU per actually grazed ha and herd size up to 50), without fertilization or with fertilization as in zone II, fallow, shrub, and forest.

In parts of the river basin up to the second pour point:

- no use of biocides,
- the possibility of divided doses of organic and mineral fertilizers to the amount recommended according to the nature of the land and the condition of the soil with regard to ensuring the protection of the aquatic environment,

- only permanent meadows with the possibility of grazing by cattle and sheep (max. 0.5 LU per actually grazed ha and herd size up to 100).

Zone I–III: In the whole SPA and the protection zone of the SPA, it is necessary to strictly exclude wintering grounds, temporary sheep pens, and assembly pens because they cause permanent damage to the soil surface, erosion, and leaching of nutrients from animal excrements into the basin. Field manure storage, silage piles, and storage of silage in silage bags are also excluded here.

Annex 12. Efficiency of low-loaded biological ponds for post-treatment in the village of Zbytiny

Figure 249 shows the change in water quality on the comparison of ammoniacal nitrogen values (strongly toxic forms of nitrogen for pearl mussels) at the outlet of the Zbytinský potok stream from the village of Zbytiny in the years before (2006–2008) and after (2008–2010) the construction of WWTP system with post-treatment biological ponds.

It is possible to see a significant decrease of ammoniacal nitrogen in the mouth of the Zbytinský potok stream to the Blanice River after the construction of post-treatment ponds in comparison with the values from 2006–2008 (before the construction of these ponds). Within the whole system, the presence of ammoniacal nitrogen in the water decreased by almost 75 % compared to the values contained in the water of the Zbytinský potok stream before water treatment introduction. **Figure 188** shows the system of these ponds.

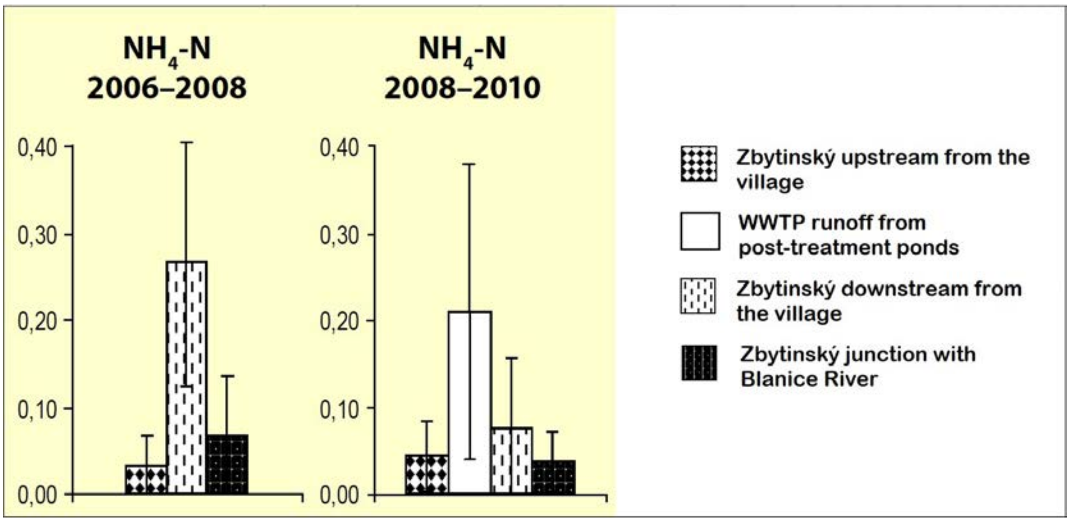


Fig. 249. Change in ammonia nitrogen value (mg/l) on different profiles of Zbytinský potok stream before and after introduction of WWTP with post-treatment ponds (see Wanner et al. 2012 for more detail).

Fig. 250. Wastewater treatment system in the village of Zbytiny with post-treatment ponds. Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

Annex 13. Overview of habitats and suitable types of management

Table 8. An overview of various types of habitats according to the Catalogue of Habitats of the Czech Republic (Chytrý et al. 2010) and suitable management methods. Habitat types significant for pearl mussel highlighted in green.

Natural habitat	Type of management				Habitat significant for pearl mussels for food reasons	Note
	none	mowing	grazing	clearing of naturally regenerated plants		
V4A Macrophyte vegetation of water streams	yes	no	no	no	yes!	enough water macrophytes in the Vltava River and Lužní potok stream
M1.1 Reed beds of eutrophic still waters	yes	no	no	no	no	rather small-scale occurrence
M1.4 Riverine reed vegetation	yes	no	no	no	no	it has spread locally a lot in recent years
M1.5 Reed vegetation of brooks	yes	no	no	no	yes	they are a rarer type of habitat in the area
M1.7 Tall-sedge beds	yes	no	no	no	no	
R1.2 Meadow springs without tufa formation	no	yes	no	yes	yes!	one of the significant habitats affecting the amount of detritus in food
R1.4 Forest springs without tufa formation	no	no	no	yes	yes	support the preservation of forest springs, do not drain forest stands
R2.2 Acidic moss-rich fens	no	yes	no	yes	yes	
R2.3 Transitional mires	yes	no	no	yes	yes	
T1.1 Mesic *Arrhenatherum* meadows	no	yes	exceptionally yes in autumn	no	no	preserved types rare in the area
T1.2 Montane *Trisetum* meadows	no	yes	exceptionally yes in autumn	no	no	only marginal in the area
T1.3 *Cynosurus* pastures	no	only additionally	yes	no	no	only degraded types in the area

T1.4 Alluvial *Alopecurus* meadows	no	yes	no	no	yes!	relatively rare, it is suitable to mow all types
T1.5 Wet *Cirsium* meadows	no	yes	exceptionally	yes	yes	
T1.5 W Wet Cirsium meadows with dominat Carex brizoides	exceptionally maybe yes	exceptionally locally yes	no	exceptionally	no	widespread vegetation
T1.6 Wet *Filipendula* grasslands	no	recommended only sometimes	no	yes	no	
T1.9 Intermittently wet *Molinia* meadows	no	yes	no	yes	yes	considerable decline in recent years
T2.3B Submontane and montane *Nardus* grasslands without *Juniperus communis*	no	rather rare	yes	yes	no	
L2.1 Montane grey alder galleries (*Alnus incana*)	yes	no	no	no	no	
L2.2 Ash-alder alluvial forests	no	no	no	locally yes	yes	
X5 – Intensively managed meadows	no	yes	yes	no	no	
X7A – Herbaceous ruderal vegetation outside human settlements, stands valuable for nature conservation	yes	mowing areas with *Lupinus polyphyllus*	no	no	no	
X12A – Stands of early successional woody species valuable for nature conservation	no	no	no	locally yes	no	

Annex 14. Photo-documentation of the development of growing fascine bundles with gravel stabilizing an inappropriately sunken, partially revitalized riverbed in the village of Zbytiny

The example of the exceptionally well-documented partial revitalization of the Zbytinský potok stream is an argument in favour of complete stream revitalizations and shows the need for a good project, including a hydraulic assessment of soil cohesion at a given slope and riverbed capacity.

Fig. 251. Zbytinský potok riverbed in 2003 – straightened riverbed sunk about 1 m below the terrain, fortified with concrete slabs over the entire area.

Fig. 252. In November 2004, partial riverbed revitalization was carried out – the concrete fortifications pulled out and meanders marked.

Fig. 253. The situation in April 2005 still shows rather sloping banks.

Fig. 254. Several large storm floods in summer 2005 led to significant lateral erosion. In the same year, as a follow-up measure to mitigate the consequences of excessive erosion for pearl mussel colonies in the main Blanice stream, a sedimentation pond was built under the revitalized section (see Figures 182 and 183 in Chapter 10.3.1).

Fig. 255. State of the banks in December 2005, when massive sand deposits into the main Blanice stream had already been recorded. As a solution, growing fascine bundles with gravel were recomended.

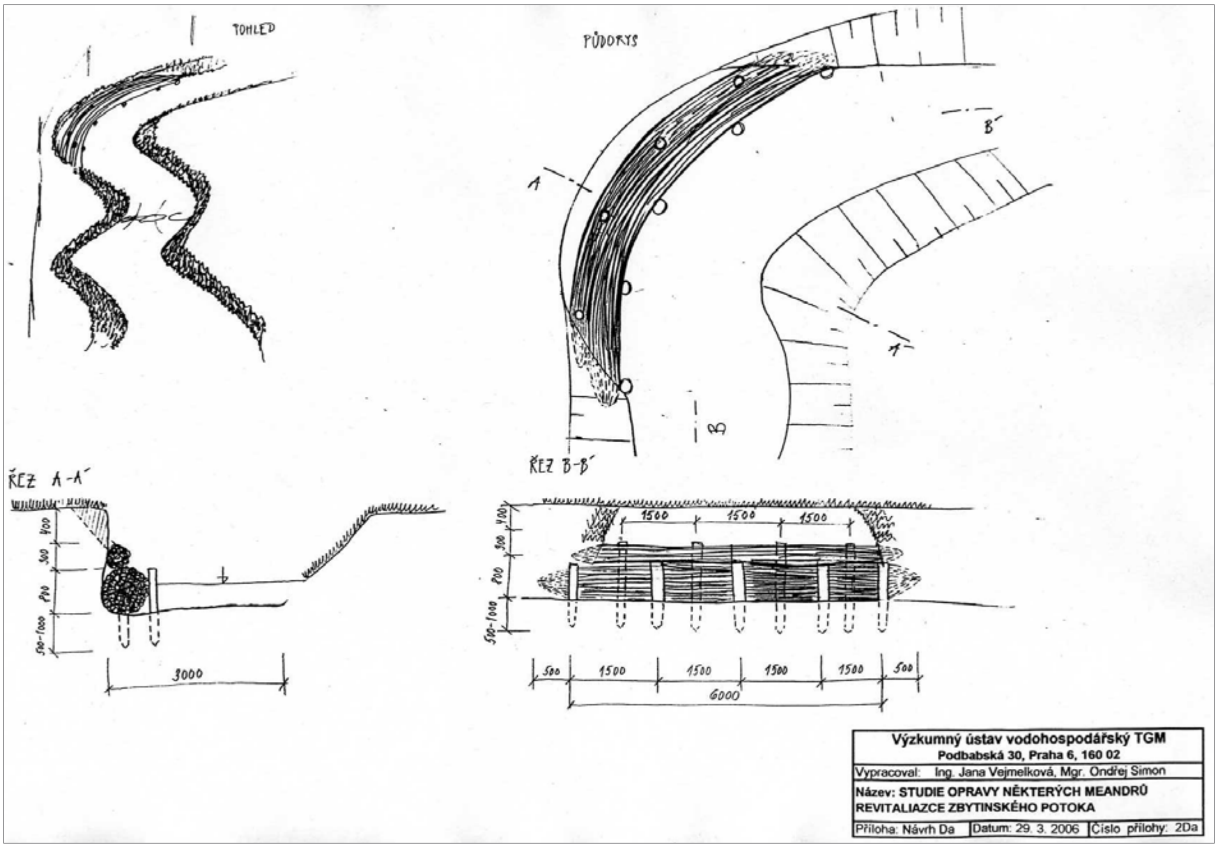


Fig. 256. Proposed solution from March 2006 for this particular location (J. Vejmělková and O. Simon).

Fig. 257. Installation of a larger number of growing fascine bundles with gravel into the stream according to the project of Vejmělková and Simon (2006), carried out in November 2006 by stream manager.

Fig. 258. The same set of two fascine bundles remediating a 150 cm high erosion wall after one vegetation season, 2007 (vegetation near the fencing was partially browsed by cows, the height of the vegetation up to 80 cm).

Fig. 259. Growing fascine bundles with gravel detail after one vegetation season, 2007 (fastening pole made of spruce log, bundles of wicker tied with burnt wire; they sprout densely and root from below).

Fig. 260. General view of Zbytinský potok stream at water level on the longest growing fascine bundles with gravel after ten years (2016).

Fig. 261. The same growing fascine bundles with gravel in 2016 as seen from the meadow.

Fig. 262. Current state (2017) of overgrown fascine bundles with gravel (in red circles). Source: Mapy.cz, © Seznam.cz, a. s., © TopGis, s. r. o.

Annex 15. Table of use of bioindications taken from the currently valid Action Plan (AOPK ČR 2013a)

Table 9. Basic types of used bioindication tests and recommended methodologies. Adapted from Dort and Hruška (2008) and the Action Plan (AOPK ČR 2013a).

	IN-situ				EX-situ
	Short-term tests	Long-term tests	Orientation tests	Tests for evaluation and research	Short-term tests
Technical parameters	Plates according to Buddensiek (10 individuals per a plate).	Plates according to Buddensiek (10 individuals per a plate), cages.	Cages, plates according to Buddensiek, other systems.	Plates according to Buddensiek, special systems.	Laboratory vessel with 5–10 juveniles (50 ml of water per one specimen, level height max. 10 mm); detritus treated by filtration (layer height max. 1 mm).
Used life cycle phase	Juvenile individual size 800–1120 µm in the second growth period (one specimen per cell in a plate).	Juvenile individual size 800–1120 µm in the second growth period (one specimen per cell in a plate).	According to the aim of a specific output, recommended juvenile individual size 800–1120 µm in the second growth period (one specimen per cell in a plate).	According to the aim of a specific output, recommended juvenile individual size 800–1120 µm in the second growth period (one specimen per cell in a plate).	Juvenile individuals larger than 1000 µm in the second growth period (5–10 specimens per plate); stable ambient temperature 18–19 °C.
Length of exposure	Various (according to the aim of a specific output), min. 5 days (Hruška 1995), recommended 30 days in summer period (7-day period of data collection).	Various (according to the aim of a specific output), recommended 90 days in summer period (14-day period of data collection).	Various (according to the aim of a specific output).	Various (according to the aim of a specific output).	Recommended 20-day period of a test with a change of detritus after 10 days.
Possibilities of use	Seasonal testing of species survival conditions (detritus trophy, watercourse toxicity) or impact of the accident or climate extreme. Evaluation of the effectiveness of management interventions.	Year-on-year testing of conditions for species survival (detritus trophy, watercourse toxicity) or impact of the accident or climate extreme. Evaluation of the effectiveness of management interventions.	Partial additional check (e.g., the effectiveness of management interventions, auxiliary check on rearing and reproductive features, measured profiles of main stream and tributaries, testing impact of the accident or climate extreme).	Long-term comparisons of the effect of biotic (e.g. life cycle stages, host influence), microhabitat (e.g. detritus trophy, interstitial conditions) and chemical-physical parameters of the environment (e.g. water temperature, exposure length, flow rate) and applied CAP management on the species life cycle.	Direct evaluation of the trophy of detritus samples (testing the conditions for species survival), evaluation of the effectiveness of management interventions.

VÚV TGM