Strategies to re-introduce *Unio crassus* and its affiliated host fish in the River Suså

Management plan (Action: A1) for UC LIFE Denmark (LIFE15NAT/DK/000948): Actions for improved conservation status of the thick-shelled river mussel (*Unio crassus*) in Denmark

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Introduction

The European LIFE project ***UC LIFE Denmark (LIFE15NAT/DK/000948): Actions for improved conservation status of the thick-shelled river mussel (Unio crassus) in Denmark*** aims to improve the status of the highly threatened thick-shelled mussel species *Unio crassus* in the River Susĺ, located on the island Zealand (Danish: Sjćlland, Fig 1). Latest mussel inventories showed, that the current population of *U. crassus* in the Susĺ river system is small and represented by old individuals scattered in the Upper Susĺ and in Torpe Kanal (Schneider and Zülsdorff, 2017a). The conservation measures intended by *UC LIFE Denmark* encompass habitat improvement in both the Upper and Lower Susĺ, where enhancement/re-introduction of *U. crassus* and two of its affiliated host fish species, the Eurasian minnow (*Phoxinus phoxinus*) and the European bullhead (*Cottus gobio*) should take place.

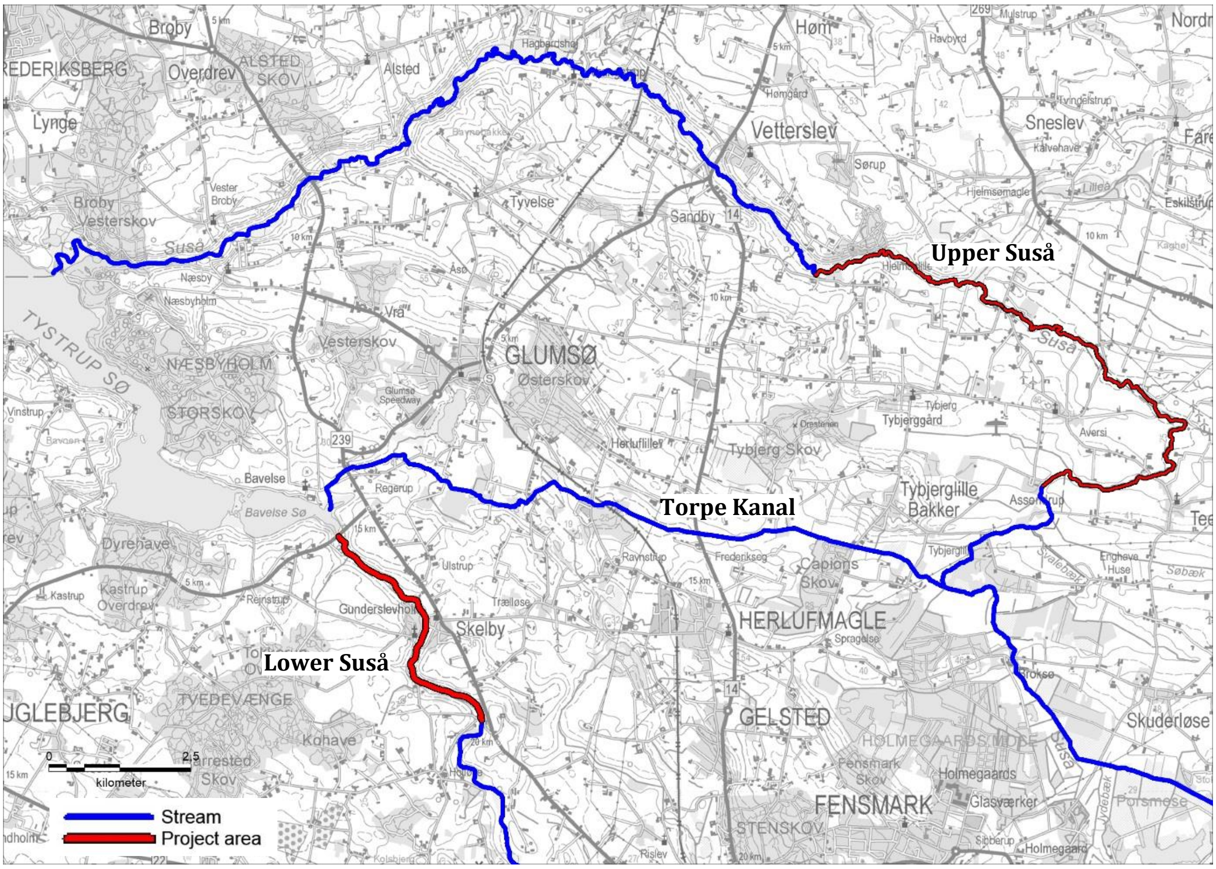

Fig. 1 Overview map of the River Susĺ. The map was kindly provided by Nćstved Municipality.

This ***management plan*** (Action: A1) is framed to compile essential information, suggestions and recommendations for successful implementation of conservation strategies targeted for *U. crassus*, *P. phoxinus* and *C. gobio* in the River Susĺ. It is based on current and available knowledge and on the European guidelines for reintroduction of species (IUCN/SSC, 2013). However, the plan should not be regarded as 'ultima ratio', as suggestions may not prove under rapid and/or extreme changes of environmental conditions. For detailed aspects of the provided information, it is recommended to consult the original research publications and reports cited in the text.

The management plan addresses the following aspects:

1. Habitat requirements and current status of the three target species *U. crassus*, *P. phoxinus* and *C. gobio*
2. Genetic and ecological considerations essential for the selection of source populations of species targeted for re-introduction in the River Suså
3. Practical implementation of conservation strategies
4. Exit strategy and alternative conservation strategies to reach the project goals
5. Cost-benefit analyses of conservation strategies
6. An assessment of river locations for species enhancement and re-introduction
7. Recommendations for habitat restoration in the River Suså
8. Overall recommendations
9. A suggested time schedule: conservation strategies for target species

1. Habitat requirements and current status of target species

Knowledge on the biotic and abiotic needs of species targeted for population enhancement and re-introduction is essential for evaluating the biological feasibility of the aimed conservation strategies (IUCN/SSC, 2013). In the following sections, the ecological niches of *U. crassus, P. phoxinus* and *C. gobio*, and their current status and threats are elucidated.

1.1 Unio crassus

The thick-shelled river mussel *U. crassus* (Order: Unionoida) is a bivalve mollusk that inhabits rivers and streams in Europe (Fig. 2). Its thick shell is name giving and derives from calcium carbonate accumulation from the water (Helama et al., 2017). Environmental conditions strongly affect the morphology of *U. crassus* shell which shows population-specific variation in shape, length and weight (Hochwald, 2001). The typical form described in species identification keys is an evenly rounded shell anterior and posterior (vonProschwitz et al., 2017). Growth rings visible on the shell can be used for age determination (Bednarczuk, 1986). However, it is often impossible to distinguish growth rings, particularly at the oldest parts of the shell near the umbo, where the shell often is corroded (Björk, 1962).

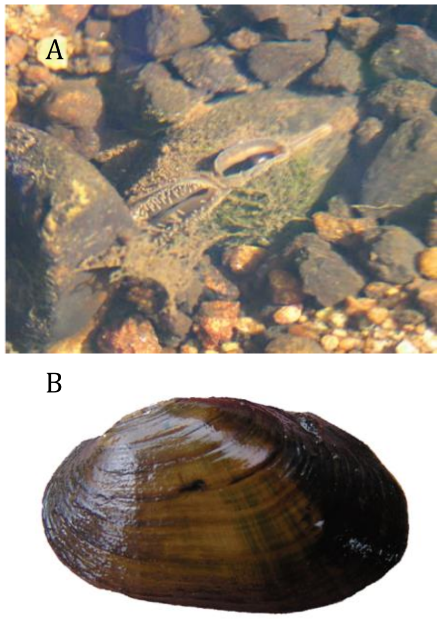

Fig. 2 (A) *Unio crassus* buried in the bottom substratum with syphons clearly visible; (B) typical shell morphology of *Unio crassus*, from vonProschwitz et al. (2017).

As freshwater mussels in general, *U. crassus* is an important ***keystone species*** in freshwaters. It provides ecosystem services (e.g. water purification, sediment mixing and stabilization) with positive effects on biodiversity and ecosystem functioning (Vaughn and Hakenkamp, 2001). However, the overall status of *U. crassus* is yet declining.

The species is categorized among the most threatened freshwater mussels in Europe (Lydeard et al., 2004). Before the industrial revolution, *U. crassus* populations were ample and dense in individuals (Trudorancea and Gruia, 1968). Mussels were fed to pigs and chicken and shell crumbles served as fertilizers on fields (Baumgärtner and Heitz, 1995). Besides species exploitation, anthropogenic land-use changes have been largely contributing to the decline of freshwater mussels worldwide (Bogan, 2008). As benthic and filter-feeding animals with long and ***complex life cycles***, mussels are particularly vulnerable to damming and channelizing of rivers, and to water pollution (Barnhart et al., 2008). Moreover, the introduction of invasive species pose additional pressure on mussel populations. For conservation of *U. crassus* in freshwaters, it is therefore essential to remove such threats and to re-establish habitats in light of the ecological niche of the species (Lopes-Lima et al., 2017).

In the following sections, the physical habitat of *U. crassus* is described and an introduction to the species' life cycle, food and feeding behavior is given. Moreover, aspects of water quality, and the status and threats of *U. crassus* are discussed.

1.1.1 PHYSICAL HABITAT

Unio crassus inhabits rivers, streams and creeks of moderate to high flow (Lopes-Lima et al., 2014; Hus et al., 2006; Zettler, 1996). Riffles, channels and pools of different water depths – from centimeters to meters, are colonized by the mussel (Bayerisches Landesamt für Umwelt (LFU), 2013). In few cases, *U. crassus* was found in lakes, particularly at lake outlets (e.g. Björk, 1962; Lundberg et al., 2006). Hence, flow velocities reported for *U. crassus* range between 0.0 m s^{-1} to 0.31 m s^{-1} (Stoeckl, 2016).

Mussel individuals live buried or partly buried in the bottom substratum, where they are relatively sedentary. Stable riverbanks consisting of ***sand*** (e.g. 0.85-2.0 mm) and ***gravel*** (e.g. 2.0-6.3 mm) are reported as the preferred habitats of *U. crassus* in many rivers (Bayerisches Landesamt für Umwelt (LFU), 2013). However, the mussel also thrives in substratum dominated by silt/clay and fine substratum (< 0.85 mm) (Buddensiek et al., 1993; Engel, 1990; Bayerisches Landesamt für Umwelt (LFU), 2013; Lundberg et al., 2006). However, high oxygen concentrations in the riverbed (interstitial) are important, hence ***redox potentials should be above 300 mV*** (Geist and Auerswald, 2007). This is due to the vertical movement of *U. crassus*, which can burry itself down to 30 cm substrate depth (Pfeiffer and Nagel, 2010; Buddensiek et al., 1993).

Mussels also move horizontally in the sediment to search and reach preferred ***microhabitats***. Such are described to differ population and river-specific. In some rivers, the middle of the river channel is colonized. In others, mussels occur at the river benches or are spread over the whole riverbed in a patchy manner. Seasonal movements between microhabitats are carried out by the mussel in both horizontal and vertical direction and are possibly linked to hydrological conditions, food availability and water temperature (Zettler and Jueg, 2007; Engel and Wächtler, 1989; Engel, 1990; Trudorancea and Gruia, 1968).

Water temperature is an important key factor for mussel growth, reproduction and survival. First, water temperature affects the metabolic rate of mussels, which generally grow faster but live shorter in geographic areas with higher temperatures (Bauer, 1992). Secondly, filtration rates are correlated with temperature. At high temperatures beyond 'normal', a trade-off between increased oxygen demands and lower oxygen concentration/saturation in the water exists. This is particularly critical for female mussels carrying brood in their gills and for mussels buried in the interstitial, where organic processes reduce oxygen (Beggel et al., 2017). Moreover, temperature affects the timing of mussel reproduction, as well as the duration and the success of juvenile mussel metamorphosis on host fish (Taeubert et al., 2014; Schneider et al., 2017b) - a life cycle stage described in the next paragraph.

1.1.2 LIFE CYCLE

As most unionid mussels, *U. crassus* has a long and complicated life cycle including a larval life stage that is a temporary ***parasite on fish*** (Taeubert et al., 2012b, Fig. 3). Mussel larvae (***glochidia***) develop from eggs kept in the female's gills (in brood pouches called marsupia) after fertilization by male sperms taken up via water filtration. In the marsupia, glochidia are bred until released to the free flowing water. The time point of glochidia release is highly triggered by temperature (Schneider et al., 2017b). After the release to the river water, glochidia attach to the gills of a host fish passing by. However, it is the fish that actively takes up the glochidia via filtration and feeding activity, as glochidia are unable to swim or move against the water current. On the fish gills, a metamorphosis of glochidia to ***juvenile mussels*** takes place. Therefore, suitable and available host fish are indispensable for the life cycle completion of *U. crassus*. Moreover, good habitat conditions are essential for successfully metamorphosed juvenile mussels,

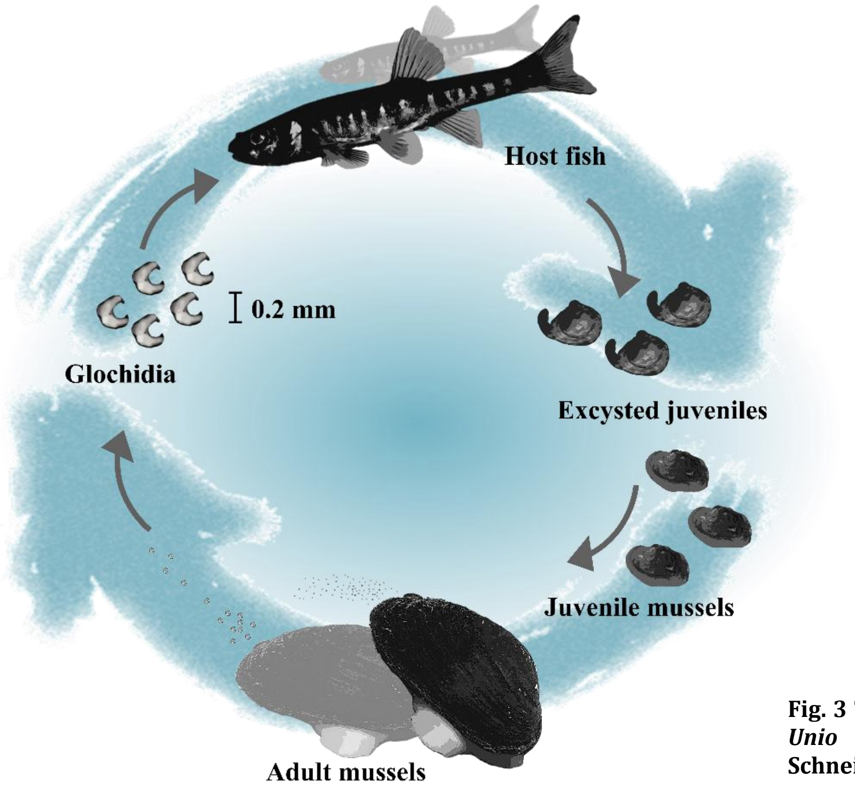

Fig. 3 The life cycle of *Unio crassus*, from Schneider (2017).

as they fall off the host fish and burry in the bottom substratum for about 3-4 years. Buried in the interstitial, juvenile mussels develop to ***adults*** (Hochwald, 1997). Survival of the juveniles is dependent on high oxygen concentrations, which is why sediments clogged with fine sediments can pose the survival of *U. crassus* at risk (Zettler and Jueg, 2007). Concluding it can be said that every life-cycle stage holds different sensitivities towards changes in habitat conditions, often caused by anthropogenic habitat disturbances (Österling et al., 2008).

Mussel reproduction takes place between the end of April and July, and occurs asynchronous in a population (Schneider, 2017; Bednarczuk, 1986). Adult mussels taking part in reproduction emerge to the sediment surface. Male mussels release sperms to the free flowing water and females take up the sperms via filtration of the gills, where eggs are fertilized in the marsupium. Reproduction events can occur up to five times per mussel individual and season (Hochwald, 1997). Hermaphroditism has only been reported in few cases (Pekkarinen, 1993). In functional populations, *U. crassus* can grow large (up to 50-55 mm in small growing populations and up to 100-120 mm in large growing populations) and old (up to 90 year, average 5- 50 years) (Helama et al., 2017; Hochwald, 2001).

1.1.3 FOOD AND FEEDING BEHAVIOR

Unio crassus thrives in streams with high contents of total organic carbon (TOC), hence where ***algae, phytoplankton, bacteria and dissolved organic matter below a size of 20 – 30 µm*** are available as food for the omnivorous bivalve (Patzner and Mueller, 2001). Feeding by adult mussels is carried out by filtering the water with up to 3.3-4.1 liters per hour and individual (Kryger and Rilisgård H.U., 1988). Filtered material not needed for own consumption is deposited to the sediment as so called ***pseudofeces*** (Strayer, 2008). In this way, mussel populations contribute largely to nutrient uptake, water purification and nutrient cycling (bioturbation) of the ecosystem. Moreover mussels stabilize sediments through their shells, but also mix sediments by means of their horizontal and vertical movements – factors positively affecting biodiversity and ecosystem functioning (Vaughn and Hakenkamp, 2001). In contrast to adult mussels, early juveniles take up food particles via cilia of their foot – a mechanism called pedal feeding (Wächtler, 2001). Gills develop in juveniles only at a later stage.

1.1.4 WATER QUALITY

In the past, *U. crassus* was categorized as highly sensitive towards nutrient loads, in particular to ***nitrate***. Threshold concentrations of nitrate nitrogen (NO_3-N) for thriving populations of *U. crassus* were set to 2.2 mg L^{-1} NO_3-N (Hochwald, 2001; Köhler, 2006; Zettler and Jueg, 2007). However, in a study of Denic et al. (2014), the mussel was found to successfully reproduce at average nitrate levels above 2.5 mg L^{-1} NO_3-N, measured in both the substratum (maximum value: 12.58 mg L^{-1} NO_3-N) and in the free-flowing water (maximal value: 15.68 mg L^{-1} NO_3-N). Moreover, NO_3-N toxicity tests on juvenile *U. crassus* resulted in categorization of the species among the least sensitive groups of freshwater mussels. "It is therefore assumed that elevated nitrate nitrogen concentrations might rather act as an indirect indicator for contamination" than being a good indicator for mussel recruitment (Stoeckl, 2016). Similar is true for ***nitrite*** nitrogen, which is released in the sediment during processes of nitrification and denitrification. One the one hand, nitrite nitrogen is assumed to have a toxic effect on juvenile mussels buried in the sediment (Buddensiek et al., 1993). On the other hand, nitrite nitrogen concentrations were found at similar levels at river locations with present and absent recruitment of *U. crassus* (Denic et al., 2014). Moreover, there is still little knowledge about ***ammonia*** sensitivities of European freshwater mussels. Results from a study on *Unio tumidus*, the painters mussel, suggest that sensitivities of adult mussels towards ammonia nitrogen may be enforced by synergistic effects of different organic pollutants (Beggel et al., 2017). Hence, it is suggested that conclusions of species-specific requirements of the chemical habitat cannot be based on mere investigation of single pollutants where sensitivities can be underestimated. It is moreover assumed, that sensitivities of early mussel life stages are considerably higher than of adults (Augspurger et al., 2007). ***Temperature*** and ***pH*** are important key parameters for mussels as they considerably affect the ion-concentrations in the water, hence biological processes and oxygen consumption (measured as Biochemical Oxygen Demand in e.g. 5 days, ***BOD_5***) of freshwaters. In Poland, the BOD_5 was found to range between 2.8-3.44 O_2 L^{-1} (average: 2.92 mg O_2 L^{-1}) in rivers where *U. crassus* exists, however at higher levels compared to rivers where mussels were absent (BOD_5 average: 2.43 mg O_2 L^{-1}; BOD_5 range: 1.9-2.8 mg O_2 L^{-1}, Hus et al., 2006). Average ***phosphate*** phosphorous concentrations of 0.04 mg PO_4–P (range 0.01-0.83 mg PO_4–P), measured by Stoeckl (2016) in a river with mussel recruitment did not vary to concentrations measured in a river where *U. crassus* was absent.

1.1.5 STATUS & THREATS

Unio crassus is classified as one of the ***most threatened freshwater mussel species in Europe*** (Lydeard et al., 2004; Lopes-Lima et al., 2017). The species is listed in annexes II and IV of the EC Habitats directive, Article 17, where the conservation status of *U. crassus* is categorized as unfavorable-bad (Eionet, 2014). In Denmark, strong population declines have been occurring since the 1990th. Originally, *U. crassus* inhabited the islands Jylland, Fyn and Sjælland. However, during the last inventory, *U. crassus* was not recorded on Jylland (Søgaard et al., 2015, Fig. 4). The extent of population decline in Denmark is hard to grasp as the national monitoring program (NOVANA) did not considered the species until the year 2000 (Larsen and Wiberg-Larsen, 2006). More information on the distribution of *U. crassus* on Fyn and Sjælland is provided in section 2.1.1 of this text.

Despite the ***high ecological plasticity of U. crassus*** in terms of habitat requirements, the benthic and filter-feeding life style of the mussel renders *U. crassus* particularly vulnerable to physical habitat disturbance and water pollution - factors that have been contributing to the drastic decline of freshwater mussel populations worldwide (Bogan, 2008).

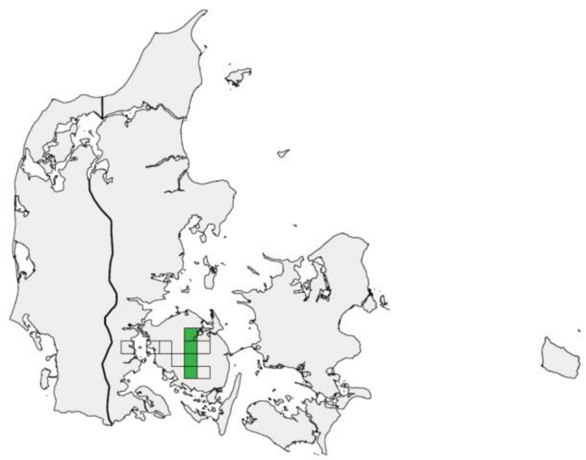

Fig. 4 Distribution area of *Unio crassus* in Denmark. Green UTM-quadrats indicate recent populations; from Søgaard et al. (2015).

Mussels and their host fish particularly suffer from ***anthropogenic land-use changes***, such as damming and canalization of rivers which change the hydrology (e.g. discharge and water flow), the temperature, as well as erosion and sediment loads of a river (Bogan, 2008; Schneider et al., 2017b; Österling et al., 2010; Vaughn, 2010). Dredging of rivers directly affects mussels as they often end up at the shores, when not collected in parallel to the measure and placed back to the river afterwards. Dredging also causes high fractions of fine sediments and organic material, which are swept downstream the river and deposit at locations with low flow. Silting and sediment clogging can lead to an oxygen lack in the interstitial at these locations and can negatively affect mussel beds and other aquatic organisms e.g. submerged plants, insect larvae (macroinvertebrates) and fish fry (Zettler and Jueg, 2007). Temporary and partially unfavorable habitat conditions such as low oxygen concentrations in the bottom substratum and high charges of fine material, may however, be tolerated by *U. crassus* (Stöckl, 2011). In contrast, fish fry of gravel spawning species including host fish species such as the Eurasian minnow (*P. phoxinus*) are more sensitive and may not survive (Mueller, 2011).

Water pollution caused by sewage discharge, runoff from excessive agriculture, animal farming and leakages of digestates from biogas fermenters is assumed one of the major reasons for the decline of freshwater mussel populations, as they also affect host fish (Poole and Downing, 2004; Beggel et al., 2017; Wang, 2007a; Bogan, 2008). Combined effects of chemical contaminants (e.g. chlorinated hydrocarbons, Sárkány-Kiss et al., 2012), metals (e.g. copper, Wang, 2007b) and pesticides (herbicides and insecticides, Bringolf et al., 2007) are deleterious.

A lack of suitable host fish is a major and direct threat to mussels, which can lead to population declines and extinctions (Strayer, 2008). In southern Germany, *U. crassus* was found to successfully recruit at host fish densities of 22-101 individuals 100 m^{-2} (average: 40 ind. 100 m^{-2}), however recruitment was lacking at fish densities less than 8 individuals 100 m^{-2} (Stoeckl et al., 2015). Besides host densities, the host composition plays a crucial role for mussel recruitment and was found to vary river-specific (Schneider, 2017). This implies that every river can hold a unique set of host fish species, which in turn depends on the local ecological conditions and the geographic area. Fish species identified as primary hosts for *U. crassus* are e.g. the European minnow (*P. phoxinus*), the European bullhead (*C. gobio*), the European chub (*Squalius cephalus*), the common bleak (*Alburnus alburnus*), and the three-spined stickleback (*Gasterosteus aculeactus*) (e.g. Douda et al., 2012; Stoeckl et al., 2015; Hochwald, 1997). However, secondary host fish such as the ruffe (*Gymnocephalus cernua*), the European perch (*Perca fluciatilis*), the burbot (*Lota lota*) and the common roach (*Rutilus rutilus*) also play an essential role for mussels populations, in supplementing or substituting primary host fish if such occur at low densities or if they are absent (Schneider, 2017).

Important to consider in conservation of freshwater mussels that are parasites on fish is their ***common evolutionary history with the fish***. Both, mussels and fish adapt to local environmental conditions including e.g. physical and chemical structures of the habitat and biological parameters such as food supply and predator presence. However, adaptations between mussels and fish also are common and driven by natural selection in the parasite-host interaction. Such can lead to fitness consequences (e.g. reduction in reproduction) of one or both antagonists (Douda et al., 2017; Schneider et al., 2017a; Stoeckl et al., 2015; Schneider et al., 2017b, 2017b). For population enhancement and re-introduction of mussels and their affiliated host fish, it is therefore suggested to test river- and species-specific host suitability prior to implementation of conservation strategies (Schneider, 2017; Stoeckl, 2016; Douda, 2015; Taeubert et al., 2012a).

Invasive species, such as the Eurasian zebra mussel (*Dreissena polymorpha*) and the Asiatic Clam (*Corbicula fluminea*) pose high threats to native mussels in freshwater ecosystems (Lydeard et al., 2004). Food-limitation, fouling and changes of benthic habitat quality are major threats related to *D. polymorpha* (Strayer, 2008). This small mussel species with origin in the Black and Caspian Sea has planktonic larvae (veliger) that can attach to all kinds of solid surfaces by means of adhesive byssal fibers. This enables fast spread and extensive invasions of *D. polymorpha* (Ricciardi et al., 1998). As for most invasive species, densities of *D. polymorpha* usually increase fastest at the early stages of invasion and drop over time with stabilization at a certain density level (Nalepa and Schloesser, 2013). The impact on the ecosystem including native mussels is assumed to follow this invasion curve.

Fig. 5 (A) The native freshwater mussel *Unio crassus* overgrown by four individuals of the invasive zebra mussel (*Dreissena polymorpha*); (B) shell findings of *U. crassus* after predation by the invasive muskrat (*Ondatra zibethicus*), which however has not yet been reported for Denmark (right), pictures from Bayerisches Landesamt für Umwelt (LFU) (2013).

Native mussels are mostly overgrown near the syphons, where *D. polymorpha* benefits from filtration currents and outcompetes the native unionid mussels for food. Hence, unionids sieged by *D. polymorpha* are starving and disabled in movement, resulting in a reduction of reproduction and death, and leading to populations decline and extinction of native mussels (Bauer and Wächtler, 2001). Regarding habitat quality, *D. polymorpha* has both positive and negative effects on the ecosystem. Positive effects are linked with water purification as mussels have high capacities of nutrient retention and biodeposition (Strayer, 2008). A reduction of phytoplankton can be observed in line with a heavy invasion of *D. polymorpha* and can lead to changes in zooplankton abundance and fish composition (MacIsaac, 1996; Strayer et al., 2004). Macrophyte abundances can increase due to improved water clearance and light penetration. However, below dense mussel beds of *D. polymorpa*, the composition of benthic organisms experiences shifts due to accumulation of organic material. Increased growth of green algae is common, as well as a loss of macroinvertebrates (including unionid mussels) important for sediment-mixing (Strayer and Smith, 2001; Lowe and Pillsbury, 1995).

In Denmark, *D. polymorpha* has first been reported in the Copenhagen area in 1840 (Andersen et al., 2009). Since then, the species has been spreading on Sjælland and has also been invading Jylland. Today, *D. polymorpha* is still largely absent on Fyn, however one location downstream of an *U. crassus* population was found inhabited by this invasive species (personal communication Palle P. Myssen, 2018). In the river Suså, *D. polymorpha* has been occurring in the lower part of the river for over 40 years (personal communication, Palle P. Myssen, 2018), but quantitative estimations of mussel density and evaluation of the ecosystem impact by this bivalve have not been conducted yet. A comprehensive report about *D. polymorpha* in Denmark, particularly on Jylland is provided by Andersen et al. (2009).

1.1.6 GENETIC EFFECTS IN DECREASING MUSSEL POPULATIONS

In mussel populations decreasing in ***effective size***, limited reproduction success is highly critical and can derive from a lack of adult mussels producing gametes, which are sperms and eggs (Mosley et al., 2014; Simberloff and Abele, 1982). Consequently, small and isolated populations can suffer from a ***loss of genetic variability*** (loss of heterozygosity, i.e. reduced variation in the amount of genetic information within and among individuals of a population). Genetic effects, such as founder effects, genetic drift and inbreeding depression play an important role herein. ***Founder effects*** can occur in populations founded from few individuals with low genetic variability and can result in limited adaptation potential to biotic and abiotic changes, and stochastic events (Hoftyzer et al., 2008). Moreover, in small populations high rates of ***genetic drift*** can occur, which is a random process of mutations leading to changes in allele frequencies within a population. This means that genetic drift can lead to loss or fixation of alleles (different forms of a gene), and hidden diseases can occur more often (Stearns and Hoekstra, 2005). ***Inbreeding depression*** can result from mating of individuals related by ancestry with loss of heterozygosity, and can negatively affect fitness (related to decreased growth rate, survival, and fecundity, Hoftyzer et al., 2008). These genetic effects can enhance the downward spiral towards extinction, termed the 'extinction vortex' (Feind et al., 2017; Berg et al., 2008; UN, 1993). To preserve unionid mussels as keystone species in freshwaters above minimum density thresholds, it is therefore essential to reduce deterministic threats to mussels and their affiliated host fish, and to re-establish habitats and connectivity in a river.

1.2 Phoxinus phoxnius

The Eurasian minnow (*P. phoxinus*) is a small (7-10 cm) ***cyprinid*** fish species. Its body shape is relatively elongated, however slightly compressed and rounded in the back (Carl et al., 2007, Fig. 6). The lateral line is obvious and extents to the anal fin. *Phoxinus phoxinus* has a blunt snout, relatively short-based fins, and inconspicuous scales (McGavin et al., 2008). As pelagic fish species, *P. phoxinus* shows a distinct shoaling behavior.

Fig. 6 *Phoxinus phoxinus*, from Carl et al. (2007).

Phoxinus phoxinus plays an important role in the food chain of freshwaters (Carl and Rask Møller, 2012). Moreover, it represents ***one of the most important (primary) host fish*** species of *U. crassus* in many European rivers. To our knowledge, no acquired immunity to mussel larvae has been observed and reported for this fish species. In contrast, larger fish individuals were found to contribute to higher numbers of successfully metamorphosed juveniles than smaller fish, albeit exposed to glochidia before (Schneider, 2017; Hochwald, 1997). *Phoxinus phoxinus* also hosts other freshwater mussel species than *U. crassus*, such as *Unio pictorum* and *Anodonta anatina* (Kekäläinen et al., 2014; Österling et al., 2017). In commercial fisheries, *P. phoxinus* has no importance. However, it is often used as baitfish in sport fisheries which has been resulting in introductions and invasions of *P. phoxinus* in e.g. Norway, where the species is now considered as a pest in multiple mountain area lakes, where originally few fish species existed (see Museth et al., 2007 and section 3.2.5 of this text).

The following sections present the physical habitat and life cycle of *P. phoxinus*, as well as its food and feeding behavior, water quality requirements, status and major threats.

1.2.1 PHYSICAL HABITAT

Phoxinus phoxinus inhabits flowing waters, ponds, shallow lakes and fringes of deep lakes. In rivers, this pelagic fish swims in gentle to **moderate flow** (0.2 – 0.3 m s^{-1}), over substratum dominated by ***stones and gravels*** (Frost, 1943; Mann, 1996). A substrate size of 16-32 mm used for river-restoration was found to be an ideal spawning ground for *P. phoxinus* (Mueller et al., 2014). However, *P. phoxinus* also occurs in habitats with silt and vegetation, but not during spawning. Moreover, microhabitat preferences of *P. phoxinus* are temperature and food dependent. In Sweden, this fish species was assigned a ***temperature*** range of 15-20 ˚C (Trigal and Degerman, 2015). In Germany, temperatures up to 26 ˚C are reported (Blohm et al., 1994). However, it was shown that minnows use warmer shallows adjacent to a river during day (shallow > 1 ˚C warmer as the main river) and the deeper, colder main river during night for feeding. This behavior was identified as avoidance of predators, which are piscivorous fish such as the brown trout (*Salmo trutta*). Over day, river shallows can be occupied by large numbers of *P. phoxinus* (> 100 ind. m^{-2}). Fish individuals returning to the main river to feed wait for shoalmates, albeit having feeding considerations (Garner et al., 1998). Similar to shallows, dead wood, overhanging river banks, tree roots and aquatic plants are important hiding places for *P. phoxinus*, particularly in the colder season, where the fish are quiescent (Blohm et al., 1994). This explains why *P. phoxinus* is generally absent from the free-flowing water during winter (Frost, 1943). In the warmer season (April to October), *P. phoxinus* is active and swims in shoals comprising hundreds or more fish individuals of all sizes and age classes, usually up to 4-5 years (Freyhof and Brooks, 2011). Segregation into age classes occurs during spawning, where adult mature fish separate from young, sexually immature minnows of 25-23 mm length and dark coloration.

1.2.2 LIFE CYCLE

The breeding season of *P. phoxinus* takes place between May and July, where fish ***migrate to their spawning grounds*** – a process highly triggered by temperature. Spawning occurs **asynchronous** in a population and at multiple times for individuals (Rasotto et al., 1987). At an age of one and about 41-45 mm of body size *P. phoxinus* become sexually mature. During the breeding season, males develop bright red, abdominal breeding coloration and tubercles (Kekäläinen et al., 2014). Female minnows are clearly attracted to this sexual ornamentation, as well as to olfactory cues of males (Lai et al., 2013). High swimming activity, twisting and turning in the current occurs. Oviposition and fertilization takes place near the stream bottom where egg masses are deposited underneath stones (Frost, 1943). After development, the fish fry moves deeper in the interstitial (up to 30 cm), where it still feeds on the yolk sack for about 10 to 15 days. Afterwards, the fish fry emerges to the free flowing water, starts shoaling similar to adults, but often seeks for shelter. In suitable habitats, the reproduction potential of *P. phoxinus* is high.

1.2.3 FOOD AND FEEDING BEHAVIOR

In streams, *P. phoxinus* mainly feeds on ***insect larvae*** and ***pupae*** of chironomids, tricoperans and ephemeropterans living in the interstitial, but the species was also found to consume adult insects and plants (Collin and Fumagalli, 2011; Frost, 1943). In lakes, *P. phoxinus* feeds on crustaceans (cladocerans) that are free-living organisms in the water column (Frost, 1943). Feeding migrations occur between day and night, and are related to predator avoidance (see section 1.2.1).

1.2.4 WATER QUALITY

As for most cyprinids, the sensitivity of *P. phoxinus* towards nutrient loads does not seem to be high, however towards ***low pH*** (Hultberg, 1977; Poléo et al., 1997). Moreover, the gravel spawning *P. phoxinus* is dependent upon ***good oxygen conditions*** in the interstitial (Frost, 1943).

1.2.5 STATUS & THREATS

According to the Red List of threatened species, *P. phoxinus* is categorized with Least Concern (Freyhof and Brooks, 2011; Kottelat and Freyhof, 2007). However, it is ***highly threatened locally*** by pollution, excessive stocking of salmonid fish species and other predators. Moreover, fine sediment loads clogging the interstitial of rivers, particularly at spawning grounds, are detrimental for successful reproduction of the species.

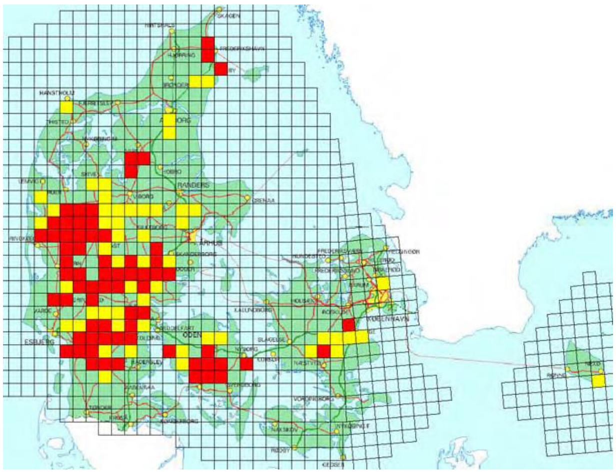

Fig. 7 Distribution area of *P. phoxinus* in Denmark. Red squares: data since 1996; yellow squares; data before 1996; from (Zoologisk Museum og Danmarks Fiskeiundersøgelser, 2007).

In Denmark, the species is categorized with Least Concern (Wind and Pihl, 2004). It occurs on Jylland, where *P. phoxinus* is largely distributed south of Limfjorden, however less in the northern parts. On Fyn and on Sjælland, the distribution of *P. phoxinus* is relatively limited (Fig. 7). Generally, strong population declines occurred in the 1990th, mainly due to water pollution. Today, population abundances are much lower than earlier reported (Carl and Rask Møller, 2012).

1.3 Cottus gobio

The European bullhead (*C. gobio*) is a small (4-15 cm) bottom dwelling (***benthic***) fish species without a swim bladder (Tomlinson and Perrow, 2003). Its large head can account for 25 % of its body length and carries two distinct eyes on the top (Fig. 8). The body flattens dorso-ventrally to the posterior end and has a mottled skin adapting to background color. In small streams, the fish can contribute substantially to total fish biomass, where it can be an **important (primary) host fish for *U. crassus*** (Schneider, 2017; Lundberg et al., 2006; Tomlinson and Perrow, 2003; Douda, 2013).

Fig. 8 The European bullhead (*Cottus gobio*), from Tomlinson and Perrow (2003).

Information about the physical habitat, the live cycle, food and feeding behaviour of *C. gobio* is provided in the following sections of this text. Moreover, aspects on water quality, status and threats are presented.

1.3.1 PHYSICAL HABITAT

Cottus gobio inhabits stony streams and margins of large rivers and lakes dominated by ***sand*** and ***gravel*** (Mills and Mann, 1983). It occurs in chalk streams and in high-altitude soft waters of **moderate to high flow** velocities ranging between 0.1 m s^{-1} to > 0.8 m s^{-1} (Tomlinson and Perrow, 2003). As solitary animal with a well developed homing instinct, *C. gobio* actively defends its territory (Mills and Mann, 1983). Only during reproduction, the fish leaves its shelter from predation under stones, tree roots and woody debris. A substrate size of 16-32 mm used for river-restoration was found to be an ideal spawning ground for *C. gobio* (Mueller et al., 2014). However, homogenous sediments of that size may not provide enough shelter for adult fish (Mills and Mann, 1983). Young of the year (YOY) are descried to use shallow, stony riffles. Backwater refuges are essential for all age classes during floods (Tomlinson and Perrow, 2003). In Sweden, most rivers inhabited by *C. gobio* hold temperatures below 15 ˚C (Trigal and Degerman, 2015). Changes in ***temperature*** can be tolerated by *C. gobio* to a certain degree, however less by populations encountering low environmental change generally (Reyjol et al., 2009). Due to the relatively low migration potential of *C. gobio* – recolonization of Scandinavia took approximately 10000 years for a 1500 km range (Volckaert et al., 2002), its potential for changes in home range, usually triggered in fish by unfavorable habitat conditions, may be significant lower compared to other migratory fish species (Reyjol et al., 2009).

1.3.2 LIFE CYCLE

Spawning of *C. gobio* occurs between February to June, depending on geographic area and water temperature (Tomlinson and Perrow, 2003). Mature males (~ older than one year) attract females in emission 'knocking' sounds and in excavating a nest under a stone suitable for egg fertilization, development and brood care. ***Parental brood care*** is common for *C. gobio* and is carried out by the male. Females (~older than 3 years) lay batches of up to 400 eggs, often in nests of multiple males to increase the chance of offspring survival. Hence, one nest can comprise eggs laid by more than one females. Brood care by the male consists of rhythmic fanning with the pectoral fin and of defending the nest against brood predators, such as competing males or caddis larvae (Mills and Mann, 1983). Longer absence of fanning leads to the eggs dying off, probably due to a lack of oxygen. Successful brood care results in fish fry consuming their yolk sac within 10 days and becoming sedentary in July and August (Tomlinson and Perrow, 2003). The life span of *C. gobio* ranges between 3-10 years and is river-specific (Mills and Mann, 1983).

1.3.3 FOOD AND FEEDING BEHAVIOR

Benthic organisms are main food targets of *C. gobio*. However, the diet of this fish species changes seasonally, as *C. gobio* choses its habitat to physical conditions rather than to food availability. In the winter, crustaceans (e.g. *Gammarus* spp. and *Asellus* spp.) are preferred food, which is insect larvae in the summer. With high vision of their large eyes, *C. gobio* is able to detect food items at dusk and night. This adaptation of nocturnal foraging is a predator avoidance strategy (Tomlinson and Perrow, 2003).

1.3.4 WATER QUALITY

Cottus gobio has high requirements to water quality and can be regarded as an indicator for good water quality (Carl and Rask Møller, 2012). In particular, ***oxygen*** is a key factor for successful reproduction, because early life stages such as eggs, are highly sensitive to a lack of oxygen (Mills and Mann, 1983). Hence, ***water pollution*** increasing the oxygen consumption of a freshwater are detrimental to *C. gobio*. Moreover, paper mill effluents to a river were found to disturb the prooxidant-antioxidant balance in the liver of adult *C. gobio* (Bucher et al., 1993). Other environmental stressors identified to negatively affect *C. gobio* are temperature changes and trace metals such as cadmium (Dorts et al., 2012a).

1.3.5 STATUS & THREATS

According to the IUCN Red List of Threatened Species, *C. gobio* is categorized with Least Concern (Freyhof and Brooks, 2011). However, in many rivers, it is ***vulnerable to population fragmentation, isolation and extinction***, as a result of ***water pollution*** and the introduction of vertical structures, such as 18-20 cm **fish migration barriers** (Tomlinson and Perrow, 2003). The species is listed in Annex II of the EC Habitats Directive, Article 17 (Eionet, 2014).

Similar to *U. crassus* and *P. phoxinus*, *C. gobio* suffers from oxygen lacks in bottom substratum clogged by fine sediments (***siltation***). Increased temperatures predicted to occur as a result of climate change, were shown to largely affect the reproduction of *C. gobio*. First, a ***temperature increase*** of 4 ˚C was found to advance the timing of spawning. Secondly, a complete reproduction failure was observed at temperature increases of 8 ˚C beyond normal, where disruption of the endocrine regulation of vitellogenesis causes changes in the timing of gonad maturation (Dorts et al., 2012b). Another threat to *C. gobio* is ***stocking of predators***, such as brown trout (*Salmo trutta*), pike (*Esox lucius*), European eel (*Anguilla anguilla*), and perch (*Perca fluviatilis*, Tomlinson and Perrow, 2003). Moreover, ***introduced species***, e.g. the signal crayfish (*Pacifastacus leniusculus*) and the native white-clawed crayfish (*Austropotamobios pallipes*), compete with *C. gobio* for habitat and food, and even feed on the eggs of *C. gobio* (Tomlinson and Perrow, 2003).

In the River Suså, where the only reported record from *C. gobio* in Denmark existed, this fish species is extinct since the 1960'. Reasons behind the extinction are directly linked with river water pollution from the 1950' and with silage wastewater runoffs, in particular (Christensen, 2010).

2. Selection of source populations for *U. crassus* and its host fish based on genetic and ecological aspects

Population genetic analyses on *U. crassus* comparing mussel populations from southern and northern Germany, and one population from Sweden revealed three divergent genetic clusters (Feind et al., 2017). The populations from northern Germany and Sweden were found to form one genetic cluster, whereas the populations from southern Germany form two additional clusters. Hence, ***genetic similarities*** occur in the northern populations, which may be related to the Weichselian glacial period, in which one common ice sheet covered northern Germany and Scandinavia. After the glaciation, *U. crassus* may have re-colonized these geographic areas from one common ancestor. With Denmark lying in between Germany and Sweden, it may be assumed that Danish mussels fall in the same genetic cluster of populations from northern Germany and southern Sweden.

However, for conservation of mussels, it is important to consider that local ***adaptation to the biotic and abiotic environment*** is common. Importantly, co-adaptations between mussels and available host fish can derive from reciprocal selection pressures in the host-parasite interaction (Galbraith et al., 2015). Hence, specific fish strains or fish species can be essential for local mussel populations (Österling and Larsen, 2013; Schneider, 2017; Douda et al., 2017). The selection of source populations for species re-introduction of the target species (*U. crassus*, *C. gobio* and *P. phoxinus*) in the River Suså therefore requires careful consideration of the **environmental background of mussels and fish**, and their ***common evolutionary history***. We here suggest that mussels and fish from the same river system, that share an evolutionary history, are used for species re-introduction in the River Suså. Moreover, similar environmental conditions should prevail in the river of source populations and the River Suså.

In the following section (section 2.1), aspects important to consider for the selection of source populations for freshwater mussels are presented and specified for *U. crassus*, based on information on the current status of *U. crassus* in the River Suså (section 2.1.1). Moreover, measures recommended to carry out prior to selection of a source population are provided (section 2.1.2), and possible source populations discussed in light of genetic aspects (section 2.1.3). The chapter on *U. crassus* is followed by a similar presentation of potential source populations for the host fish species *P. phoxinus* and *C. gobio* (sections 2.2 and 2.3). Moreover, a decision tree showing multiple alternatives of source populations for all target species (*U. crassus*, *P. phoxinus* and *C. gobio*) is presented in Figure 10. The decision tree is based on potential results of population genetic analyses comparing mussels from Sælland, Fyn and southern Sweden (Skåne).

2.1 Freshwater mussels

Genetic guidelines for freshwater mussel conservation recommend to "use broodstock from the closest adjacent watershed based on stream distance and with the most similar genetic and ecological characteristic. Source populations should be similar to the recipient population based on: ***(1) genetic lineages***; ***(2) life history pattern***; ***(3) ecology of the originating environment***, and ***(4) physiographic division***" (McMurray and Roe, 2017). Moreover, source populations must hold ***high genetic variability*** to avoid genetic effects, such as founder effects, genetic drift and inbreeding depression (see section 1.1.6 of this report), in both source and founder populations.

The ***population size of the source population*** is another important parameter to consider when choosing source populations for species re-introduction and should include estimations of the total number of individuals and the proportions of breeding females (McMurray and Roe, 2017). The source population must be large enough to withstand a removal of brood used for artificial infestation of fish with glochidia, or of adult individuals used for mussel translocation. It is recommend that less than 5 % of the source population is affected by the measures (McMurray and Roe, 2017). Moreover, it is suggested to return parts of the brood used for artificial fish infestation to the source population, together with the adult individuals collected for glochidia acquisition, if not translocated to the recipient river. In this way, shortage of recruits in the wild, hence disadvantageous genetic effects in decreasing populations, is lessened (Hoftyzer et al., 2008). Importantly, mussels returned to the wild should be placed back at the locations where they were collected. Otherwise, risk for outbreeding depression (fitness reduction of offspring deriving from mating of genetically distant parent individuals where at least one parent is not adapted to local biotic and abiotic conditions) may occur.

Considerations of the ***size of the founder population*** is essential to maintain the within-population genetic variation on a long term. As mentioned above (section 1.1.6), a loss of genetic variation due to e.g. inbreeding of few founder individuals and genetic drift can result in reduced population fitness and adaptation potential to changing environmental conditions (McMurray and Roe, 2017). Guidelines for broodstock size are yet based on mathematical models, as little is known about the reproductive biology of mussels (e.g. fertilization success, sex ratios), but propose 20-25 randomly collected mussel individuals to represent ~98 % of the genetic variation in a population (Jones et al., 2006). However, large source populations (n > 5000) generally hold high levels of genetic variation, which is why higher numbers of source population individuals are recommended for re-introduction measures. A number of > 50 gravid mussels should be targeted, with individuals collected annually from different sites of the source population (Jones et al., 2006).

2.1.1 CURRENT STATUS OF *U. CRASSUS* IN THE SUSÅ RIVER SYSTEM

The latest mussel inventory of *U. crassus* in the River Suså resulted in six genetically approved individuals (Schneider and Zülsdorff, 2017a). All individuals were at high age (Fig. 9). In Torpe Kanal, a number of six individuals (not genetically approved) were found in an one day investigation (Schneider and Zülsdorff, 2017b). Here, one mussel individual carried non-developed brood in form of eggs in its gills. It may be assumed that further *U. crassus* individuals are present in the Suså river system, though the outcome of an extended search may be very low (see section 2.1.2).

Fig. 9 Exemplars of *Unio crassus* from the River Suså tagged with an individual number and a Passive Integrated Transponder (PIT), from Schneider and Zülsdorff (2017a).

2.1.2 RECOMMENDATIONS PRIOR TO SELECTION OF A SOURCE POPULATION

So far, little is known on the current population size of *U. crassus* at deeper areas of the River Suså, and in Torpe Kanal, where few effort was made since the last mussel inventory in 2014 (Schneider and Zülsdorff, 2017a; Schneider and Zülsdorff, 2017b). We therefore recommend an ***extended search* for *U. crassus*** in the Upper Suså (e.g. near Vrangstrup) and in Torpe Kanal (e.g. near Holmen, Tingbro and Vej till Bavelse, where > 20 shells were found at 100 m, Bangsgaard Natur&Miljørådgivning, 2014). Moreover, an overview investigation for *U. crassus* in the Lower Suså could be carried out by diving. An extended search of mussels may increase the number of *U. crassus* individuals which could be pooled with mussels detected during previous investigations. By this means, the chance for successful egg fertilization in females via male sperms is increased. However, mixing of individuals between rivers is not recommended until population genetic analyses confirmed the mussels from the River Suså and Torpe Kanal to belong to one genetic population. Although, the extended search of mussels may be low in outcome (< 20 mussel individuals) it is important to enhance knowledge on the current status of *U. crassus* in the Suså river system. Moreover, we highly encourage incorporating Torpe Kanal in the national monitoring program NOVANA.

Sampling of DNA from mussels from Torpe Kanal and from Fyn (Odense Å and Hågerup Å) is recommended to confirm the species *U. crassus* yet identified morphologically, also genetically. This has already been done for mussels collected in Suså, but may be extended for individuals potentially found during additional investigations. Moreover, ***population genetic analyses*** comparing the remaining mussel population in Suså with alternative source populations from Denmark (Fyn) and from southern Sweden (Region Skåne) are highly recommended. This enables evaluation of the genetic background of potential source populations in terms of genetic variability and similarity to mussels from Suså. The analyses may also resolve the question whether mussels from Torpe Kanal and from Suså can be defined as one common genetic population.

If no population genetic analyses can be carried out, the selection of a source population should largely be based on an ***analysis of similarity of environmental conditions*** between rivers of potential source populations and the River Suså. Moreover, potential risks for source and founder populations should be evaluated (IUCN/SSC, 2013). The population with the most similar environmental background and **least *risk for source and founder populations*** should be chosen as broodstock. This encompasses that a source population should be large enough to allow for genetic variance. Moreover, no migration barrier isolating mussels, fish and other aquatic organisms should occur in close proximity of the population. Finally, a variety of adult mussel individuals should be used for artificial infestation of host fish with mussel larvae (see section 2.1). The selection of a source population and river locations from which mussels are collected should be carried out in agreement with local specialists and the environmental protection agency.

2.1.3 POTENTIAL SOURCE POPULATIONS – *U. CRASSUS*

Conservation of *U. crassus* in Suså in form of population enhancement (source population = founder population, IUCN/SSC, 2013) is not recommended because of multiple reasons. First, it is yet unknown whether successful brood fertilization in females via male sperms occurs in the River Suså. Although mussels from Suså were aggregated after the last inventory in the river, the probability for successful reproduction is low because the mussel population is small, old and was originally scattered. Secondly, it is yet unclear whether mussels from Suså and Torpe Kanal represent one or two isolated genetic populations. However, this is important information for evaluating whether merging of mussels from both rivers is feasible. Merging would increase the number of founder individuals and hereby the chance for successful brood fertilization. Nevertheless, merging of two isolated populations can lead to outbreeding depression with negative fitness consequences. Thirdly, there is a high risk for genetic effects in small founder populations which can suffer from founder effects, inbreeding depression and genetic drift (see section 1.1.6). The risk greatly depends on the actual population size, including proportions of females taking part in reproduction, and the genetic variance of the founder population. As mentioned above, it is therefore recommended to carry out population genetic analyses revealing the level of genetic variance in mussels from the Suså system and facilitating the selection of a suitable source population.

Potential source populations for re-introduction of *U. crassus* that are geographically closest to the River Suså are located on Fyn (e.g. rivers Odense Å and Hågerup Å; Bangsgaard Natur&Miljørådgivning, 2014) and in southern Sweden (e.g. rivers Bråån and Tommarpsån; Schneider et al., 2017a). On Sjælland, no other *U. crassus* rivers than Suså and Torpe Kanal are currently known. To test whether mussels from Odense Å and Hågerup Å are genetically similar to mussels from Suså, hence genetically suitable source populations, we suggest to include such mussel rivers in population genetic analyses. Results may reveal that one or two specific population are most similar to mussels from Suså. However, results may also show no significant difference between the populations tested (Fig. 10). If the latter is true or if no population genetic analyses can be carried out, the selection of a source population must be greatly based on ecological parameters (see section 2.1).

In the following sections, information on population densities and trends, self-recruitment of populations and possible risks for source and founder populations are presented and discussed for mussel populations from Fyn and Skåne. In southern Sweden, there are further rivers with thriving *U. crassus* populations, however little is known about their present recruitment status and their relationship to host fish, hence are not further addressed. However, more information can be obtained from e.g. vonProschwitz et al. (2017) and Svensson and Ekström (2005).

On Fyn, the River ***Odense Å*** holds about 95 % of Denmark's *U. crassus* on a long river stretch of 36 km (Søgaard et al., 2013; Larsen and Wiberg-Larsen, 2006). Thereof, most mussels were reported for a 6 km river stretch that is unregulated (Larsen and Wiberg-Larsen, 2006). Overall, the population mainly consists of old individuals. The estimated population size of *U. crassus* is 40000 individuals and densities range between 0.05-0.92 ind. m^{-2} (Bangsgaard Natur&Miljørådgivning, 2014). It is believed that the population once was about 80 % larger, and may have reduced to the actual size during only 3-4 years (Søgaard et al., 2013). A European LIFE project (LIFE REGAIN – Regional actions to improve nature in River Odense and Odense Fjord, LIFE04/NAT/DK/000022) carried out habitat restoration and rehabilitation in the river. *Unio crassus* was one of the target species in the project. Post-restoration, it is assumed that the conservation status of the mussel improved due to the conservation measures conducted (Lindberg Birkelund et al., 2010). Recently, one mussel individual of 30 mm was discovered, indicating recruitment in the population, even if at very limited extend (Søgaard et al., 2015). However, based on current knowledge, it is yet unclear whether a removal of brood needed for artificial fish infestation poses the wild population at risk. A risk analysis is therefore recommended. If a removal of brood is evaluated to not be critical for self-recruitment of mussels in Odense Å, the population is feasible as source population. However, it is recommended to return adult females picked for glochidia collection and artificial host fish infestation to the river. Moreover, the mussels should be maintained in river water from Odense Å to not spread or introduce potential parasites or diseases to Odense Å when returning the mussels. Additionally, handling of mussels (e.g. transport and maintenance) should be carried out with care and at lowest stress levels possible. An alternative source population from Fyn inhabits the River ***Hågerup Å*** for which mussel beds are reported to occur at 8 km of the river. The population size is estimated to 11000 individuals and average densities are reported to range between 0.16-0.62 ind. m^{-2} (Bangsgaard Natur&Miljørådgivning, 2014; Larsen and Wiberg-Larsen, 2006). A broad age distribution of mussels is known for the population and successful mussel recruitment is assumed (Miljøministeriet and Naturstyrelsen, 2013). The river is a tributary to Odense Å. In both rivers, *P. phoxinus* is reported to occur (Søgaard et al., 2013), although at low densities (see section 2.2.1).

In the region of Skåne, two rivers (Bråån and Tommarpsån) have been in focus of much conservation work of a European LIFE project (LIFE10 NAT/SE/000046 – The thick-shelled river mussels brings LIFE+ back to rivers) and research on the relationship between *U. crassus* and its host fish *P. phoxinus* and *C. gobio* (Schneider, 2017). Re-introduction of *U. crassus* to two habitat restored rivers (Klingavälsån and Fyleån) has been conducted in using Bråån and Tommarpsån mussels as source populations. In the River ***Bråån***, a self-recruiting mussel population exists with average mussel densities of 6.2 ± 5.2 ind. m^{-2} at some river parts (Schneider et al., 2017a). *Phoxinus phoxinus* represents the primary host fish for *U. crassus* in this river, however *C. gobio* is absent (see section 2.2.3). A population genetic analysis showed that the genetic variance of *U. crassus* is high in Bråån (Feind et al., 2017). In the River ***Tommarpsån***, a self-recruiting mussel population exists with average mussel densities of 4.3 ± 5.1 ind. m^{-2} at some river parts (Schneider et al., 2017a). *Phoxinus phoxinus* and *C. gobio* represent the primary host fish species for *U. crassus* in this river (see section 2.2. and 2.3). The mussel populations in Bråån and Tommarpsån are evaluated as stable and temporary removal of gravid mussels for glochidia collection is not regarded as risk, when mussel collection and handling is carried out as described by Schneider et al. (2017a). Importantly, equipment from Denmark should be disinfected prior to entering the river, as introduction of the invasive zebra mussel (*D. polymorpha*) and diseases to the river should be avoided. Any measure should be in agreement with the County Administration Board of Skåne (Länsstyrelsen Skåne) and the Swedish Board of Agriculture (Jordbruksverket).

2.2 Phoxinus phoxinus

2.2.1 CURRENT STATUS OF *P. PHOXIUS* IN SUSÅ

The national monitoring program (NOVANA) has no records of *P. phoxinus* in the River Suså, neither was the species caught during a recent fish inventory in Suså (Gørtz and Mouillet, 2017). In Torpe Kanal, *P. phoxinus* is known to occur (Søgaard et al., 2013). However, density data is lacking from NOVANA, as Torpe Kanal is not incorporated in the program. In 2010 and 2013, two fish investigations conducted by Ringsted Produktionshøjskole and the Technical University of Denmark (DTU) recorded several (about 10) individuals of *P. phoxinus* near Tingbro. However, no fish individuals were caught in investigations in 1997, 2005 and 2015, the latter was conducted by Limno Consult (Henriksen, 2015). In the River Lynge Bæk, which is part of the Suså river system, quantitative electrofishing conducted in the year 2010 revealed densities of *P. phoxinus* of 1 ind. 100 m^{-2}. In Køge Å, which is located north-east of the Upper Suså, *P. phoxinus* occurred at densities of 4 ind. 100 m^{-2} in 2010 (data was kindly extracted from the database (NOVANA) by Peter Wiberg-Larsen, Institute for Bioscience, Aarhus University). In June 2017, a number of several 50 *P. phoxinus* individuals were fished on a 100 m stretch in Køge Å during an inventory (personal communication Peter W. Henriksen).

2.2.2 POTENTIAL SOURCE POPULATIONS – *P. PHOXINUS*

Based on the ***low abundance of P. phoxinus in the Suså system*** (incl. Torpe Kanal), it is not recommended to use these rivers as source population for fish stocking and conservation measures for *U. crassus*. First, fishing and removal of fish for breeding may pose the remaining wild population and its affiliated species (e.g. *U. crassus*) at risk. Secondly, small founder populations can suffer from genetic effects (founder effects, inbreeding depression and genetic drift). Thirdly, it is yet unknown whether *P. phoxinus* from the Suså system hold a high level of genetic variation, which however is essential to know for species conservation measures (IUCN/SSC, 2013). It is therefore recommended to carry out population genetic analyses revealing the level of genetic variance in *P. phoxinus* from the Suså system, which also is essential for the selection of alternative source populations. However, if results from population genetic analyses on mussels and fish do not suggest otherwise, there is reason to use fish from southern Sweden, as *C. gobio* only occurring in Sweden, is supposed to be introduced to Suså. This implies that Swedish *P. phoxinus* that co-existed with *C. gobio* and *U. crassus* may be used for the conservation measures planned in Suså. In the following sections, potential source populations form southern Sweden and from Fyn are presented, and aspects regarding population densities and risks for source and founder populations discussed.

If Swedish *U. crassus* are used for population enhancement in Suså, it is recommended to introduce this species together with its affiliated host fish species. Choosing the river ***Tommarpsån*** may be wise due to the co-occurrence of *U. crassus*, *P. phoxinus* and *C. gobio*. In Tommarpsån, *P. phoxinus* was reported to occur in all age classes and at average densities of 145 ± 167 ind. 100 m^{-2} (Schneider et al., 2017a). Hence, a removal of *P. phoxinus* (total number = 600; n = 300 for rearing and n = 300 for translocation to Suså) needed for implementation of the conservation strategy, does not pose the local fish population as risk, if fishing is carried out at multiple points in time and locations in the river. Similar is true for *P. phoxinus* in the River ***Bråån*** for which average fish densities of 149 ± 100 ind. 100 m^{-2} are reported (Schneider et al., 2017a). However, *C. gobio* is not present in the River Bråån. For both rivers, fish removal is recommended to be carried out prior to the mussel reproduction season, ranging between April and July, to avoid catching of fish naturally infested with *U. crassus* glochidia. Importantly, equipment from Denmark should be disinfected prior to entering the river, as introduction of the invasive zebra mussel (*D. polymorpha*) and diseases to the river should be avoided. Any measure should be in agreement with the County Administration Board of Skåne (Länsstyrelsen Skåne) and the Swedish Board of Agriculture (Jordbruksverket).

Fish from Fyn are recommended for breeding and release of *P. phoxinus* to Suså, if mussels from Fyn are used for re-introduction in the River Suså. On Fyn, fish densities are higher than on Sjælland (see section 2.2.1), with Hågerup Å holding slightly higher *P. phoxinus* abundances (average densities: 6.69 ind. 100 m^{-2}; range: 2.86-12 ind. 100 m^{-2}) than Odense Å (average densities: 1.72 ind. 100 m^{-2}; range: 0.03-3.71 ind. 100m^{-2}, data was kindly extracted from the database (NOVANA) by Peter Wiberg-Larsen, Institute for Bioscience, Aarhus University). However, a removal of 600 fish from one of these rivers may cause high risk to the wild fish population, as well as for their affiliated mussel populations. Therefore, a *P. phoxinus* source population from Fyn is not considered feasible, when carried out according to the planned conservation strategy. However, if bred fish are released to the river as young of the year or as one year old fish infested with mussel larvae - a measure that can be regarded as compensation for fish removal, a *P. phoxinus* source population from Fyn may be feasible. Nevertheless, the compensation measures must make up for the removal of fish from the rivers taking into account the risk posed to the wild population. This means that higher numbers of fish must be released to the wild than removed at first. Fish populations from rivers on Fyn other than Odense Å and Hågerup Å, are not recommended as source populations, if the possibility to re-introduce mussels and fish from the same river system to Suså is given.

2.2.3 RECOMMENDATIONS FOR SELECTION OF A *P. PHOXINUS* SOURCE POPULATION

As mentioned above, ***population genetic analyses*** comparing fish from Sjælland, Fyn and southern Sweden are recommended. If no population genetic analyses can be carried out, the selection of source population should be based on ***ecological parameters***, such as geographic proximity, population size and status allowing for genetic variance, similarity in environmental conditions of source population rivers and Suså. Moreover, source populations should not derive from river locations at which migration barriers for fish and other aquatic organisms are in proximity of the source population. The selection of a source population and of river locations should be carried out in agreement with local specialists and the environmental protection agency.

2.3 Cottus gobio

2.3.1 STATE OF THE ART

Since the 1960th, *C. gobio* is extinct in Denmark. The species' former distribution area is assumed to be limited to the Lower Suså, where it occurred near Holløse Mølle and Herlufsholm (Carl and Rask Møller, 2012). Fish exemplars are preserved at the Natural History Museum of Denmark (Bollerup, 2015). No genetic analyses have been conducted yet. Results from genetic studies conducted in other European countries suggest that differrent lineages of *C. gobio* exist in its European distribution (Kontula and Väinölä, 2001; Volckaert et al., 2002). In Scandinavia, these lineages are assumed to have derived from different post-glacial colonization events, possibly linked with the current Würm glacial (Volckaert et al., 2002). Colonization of southern Sweden is suggested to origin from the south, possibly the river Elbe in Germany (Hanfling et al., 2002; Hänfling and Brandl, 1998). With Denmark located between Germany and Sweden, the Danish *C. gobio* may therefore derive from the same river system and cluster with the same lineage.

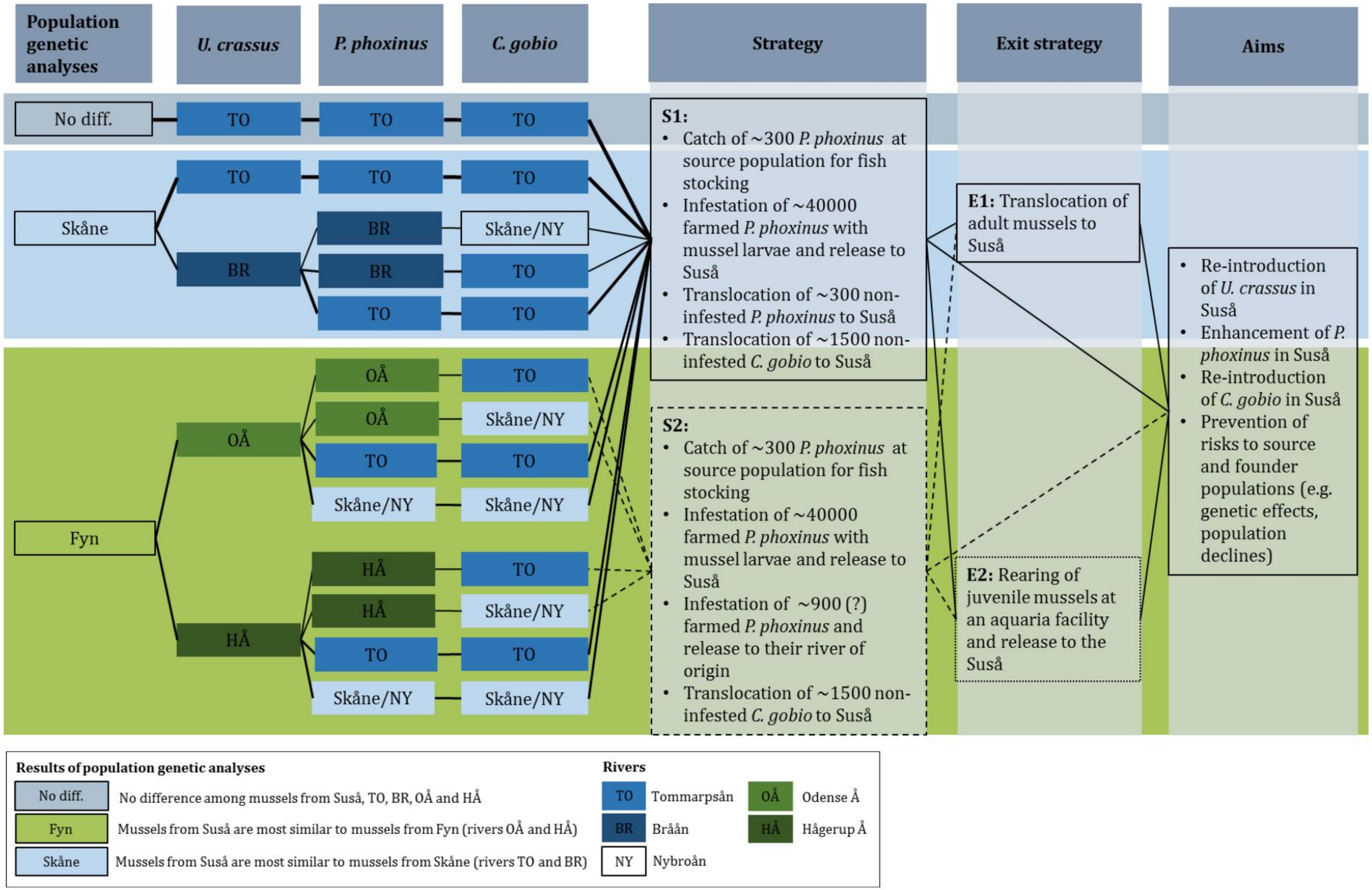

Fig. 10 Decision tree for selection of source population rivers for re-introduction of target species (*Unio crassus*, *Phoxinus phoxinus* and *Cottus gobio*) in the River Suså. Proposed suggestions for source population rivers and conservation strategy are based on possible results from population genetic analyses.

2.3.1 POTENTIAL SOURCE POPULATIONS – *C. GOBIO*

Introduction of *C. gobio* from southern Sweden to the River Suså, represents a feasible way to re-introduce the species in its former distribution area in Denmark (Bollerup, 2015, personal communication with Peter G. Hansen, DTU). We recommend to re-introduce *C. gobio* in Suså together with its affiliated mussel (*U. crassus*) and fish species (*P. phoxinus*). To this end, source populations from the river Tommarpsån, in which *U. crassus*, *P. phoxinus* and *C. gobio* co-occur, may be used. To our knowledge, there is no other river system in southern Sweden, in which the three species co-exist. *Cottus gobio* was reported to occur in Tommarpsån at average densities of 195 ± 104 ind. 100 m^{-2} (Schneider et al., 2017a). Hence, a removal of *C. gobio* (375 individuals yearly over 4 years; total number = 1500) is not considered as risk for the local fish population in Tommarpsån, if conducted at different time points between 2018-2021 and at different river locations (personal communication Anders Eklöv, fiskevard.se). An alternative source population of *C. gobio* from southern Sweden exists in Nybroån, holding high *C. gobio* abundances. However, this population may only be used when *C. gobio* is the only target species taken from Sweden.

2.3.2 RECOMMENDATIONS FOR *C. GOBIO* PRIOR TO CONSERVATION ACTION

The relative low number of *C. gobio* individuals assigned for re-introduction to the River Suså (total number = 1500) renders this fish species a valuable target species for Denmark. Therefore, a conservation approach with overall high success-probability for re-introduction should be used. Such may exclude the infestation of *C. gobio* with mussel larvae prior to its translocation to Suså, as this poses stress to the fish in addition to the translocation itself. One could argue that similar may be true for *P. phoxinus*, however, this fish species is supposed to be introduced to Suså in much higher numbers (~ 40300 individuals).

We further recommend to transfer *C. gobio* from a source population to the River Suså only after the reproduction season of the fish, and also after local mussel populations released their larvae to the free flowing water, i.e. after a potential glochidia infestation (see section 3.3.1). Importantly, equipment from Denmark should be disinfected prior to entering the donor river, as introduction of the invasive zebra mussel (*D. polymorpha*) and diseases should be avoided. Any measure should be in agreement with the County Administration Board of Skåne (Länsstyrelsen Skåne) and the Swedish Board of Agriculture (Jordbruksverket).

3. Practical implementation of conservation strategies

Successful conservation of species requires careful planning of conservation strategies. This implies that the timing and the methods used for implementation are well thought-out. For re-introduction of *U. crassus* and its host fish species *P. phoxinus* and *C. gobio* in the River Suså, there are five major project parts, that must be coordinated and matched temporally. The first part is ***habitat restoration***, which will be conducted in the Upper and Lower Suså, in particular. The second part encompasses ***farming of P. phoxinus*** at a fish hatchery, from which fish delivery must match the timing of the third and fourth parts, which are the ***collection of mussel larvae*** essential for ***artificial infestation of the fish*** and ***the subsequent release of the fish to Suså***. The fifth part encompasses the catch of ***C. gobio*** at a source population and the ***re-introduction*** of the species in Suså.

In the following sections, methods for the practical implementation of conservation strategies (parts three, four and five) are suggested and presented in detail (sections 3.1, 3.2 and 3.3).

3.1 Collection of glochidia for artificial infestation of host fish

Prior to artificial infestation of host fish, viable mussel glochidia must be collected from mussel females taking part in reproduction. This requires collection of gravid mussels in the field and transport of the mussels to a laboratory/aquaria facility, where mussels are maintained for 1-2 weeks and released glochidia collected and quantified. Usually, gravid *U. crassus* release glochidia asynchronous in a population, which is why the glochidia collection in the lab can range from several days to 1-2 weeks. The time period is depended on the number of gravid mussels collected and transferred to the aquaria facility, and the number of female parents from which glochidia are used for artificial infestation of host fish. The higher the number of gravid mussels in the lab, the higher is the probability for simultaneous glochidia releases by multiple mussels. If fish infestation is conducted at multiple time points during the reproduction season of *U. crassus*, fewer gravid mussels are required for one infestation event. One the one hand, this procedure enables stepwise infestation of fish delivered from the fish hatchery (~10000-15000 fish yearly), and may facilitate logistics. Moreover, the successive removal of few gravid mussels from a source population may affect natural reproduction to a lower degree than if high numbers of broods are removed at one event. On the other hand, missing out fish infestations at the later end of the mussel reproduction season due to a lack of gravid mussels, poses the conservation action at risk. Therefore, careful planning is required to match fish infestation and logistics with the phenology of *U. crassus*. Below, the workflow of brood collection from gravid mussels is presented in detail.

3.1.1 COLLECTION OF GRAVID MUSSEL IN THE RIVER

Gravid mussels are collected at a source population by wading through the river using aquascopes (Fig. 11A). Mussels are picked from the sediment surface by hand or by means of grabbers. No digging in the sediment is needed as mussels taking part in reproduction emerge to the sediment surface. In this way, female mussels render possible egg fertilization by sperms. Moreover, female mussels keeping brood in their gills improve oxygen uptake (Bauer, 2001). Mussels picked from the sediment surface can be temporarily stored in mesh bags placed in the river or in buckets (e.g. 10 L) set on land. Mussels transferred to buckets should be supplied with oxygen and river water of constant temperature.

Fig. 11 (A) Search for mussels using an aquascope (picture taken by Valentina Zülsdorff ©UC*for*LIFE); (B) adult gravid *Unio crassus* carefully opened using special tongs, from Schneider (2017).

Collected mussels are then visually inspected for brood in their gills. Brood is visible by a swollen and white or orange mass in the marsupium, when the mussel shell is slightly opened (< 0.5 cm) using special opening tongs (Fig. 11B). Gravid mussels are transferred to small plastic containers fit with stream water and oxygen supply. For stabilization of mussels during transport to the laboratory, it is recommended to wrap the mussels in a net (e.g. 4 mm). Stabilization of mussels reduces stress and hereby the risk of spontaneous glochidia releases. Cooling blocks can be used to keep water temperatures constant and at levels similar to the river water temperatures. Hence, temperature measurements in the river are essential. The described method has proven suitable for the transport of mussels from the field to a laboratory with purpose of artificial fish infestation (Schneider, 2017). Information of other transport methods proven suitable are described e.g. by Taeubert et al. (2012a) and Hochwald (1997).

In the laboratory, it is recommended to maintain mussel in river water collected in parallel to the search for gravid mussels. River water can be transported in plastic barrels and with oxygen supply, if transport is long and air temperature high.

3.1.2 MAINTENANCE OF MUSSEL IN THE LABORATORY

In the laboratory, mussels are maintained in aquaria fit with river water and oxygen supply. Temporary absence of substratum in aquaria facilitates glochidia collection and has shown to not be problematic for the mussels. However, water temperatures should be similar to the water temperatures at which the mussels were transported. Over time, water temperatures can be slowly increased to provoke glochidia releases. However, maximum temperatures should not exceed the range of natural river temperatures. Daily investigation of glochidia release (Fig. 12A) is recommended, preferably conducted in the morning, together with water exchange and cleaning of aquaria. Mussels should not experience sudden changes in water temperatures as this causes stress for the animal and may provoke abortion of the brood (Fig. 12B).

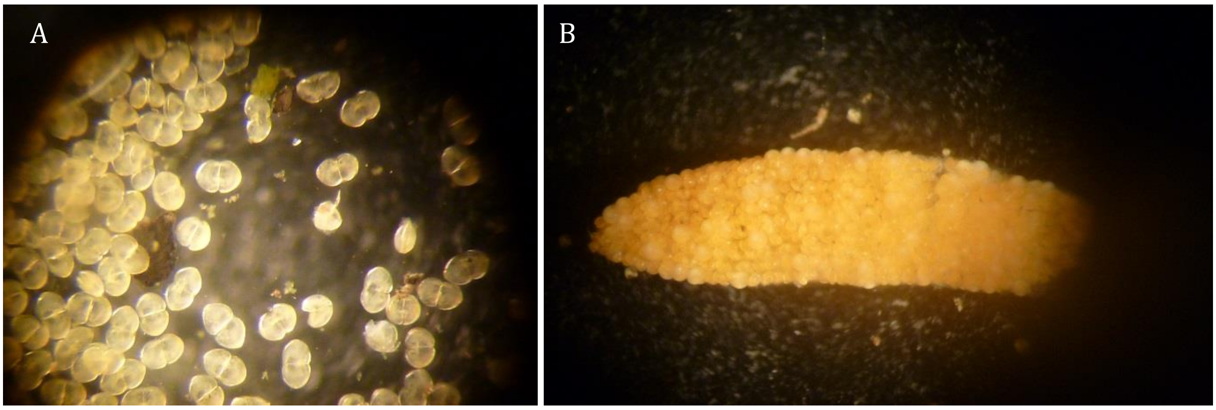

Fig. 12 (A) Glochidia of *Unio crassus*; (B) egg package of *U. crassus*; pictures taken by Lea D. Schneider ©UC*for*LIFE.

3.1.1 GLOCHIDIA COLLECTION

Released glochidia are collected by means of e.g. 50 µm sieves. A subsample of glochidia is transferred to Petri dishes for investigation of glochidia viability by using a microsope binocular and adding a drop of saturated NaCl solution (Fritts et al., 2014). Glochidia carrying out valve movements (clapping and closing of shell) are considered viable. Glochidia from several adults can be pooled and stored in beakers filled with stream water and labelled with date of release and information about the number of female parents. Glochidia can be stored at 4-8 °C for several days until fish infestation, but should be used as soon as possible post-release (Schneider et al., 2017a; Taeubert et al., 2012a).

After the release of glochidia, adult mussels (if not used for translocation to the River Suså) should be acclimated to river water temperatures and returned to the river locations at which they were collected. If a translocation is planned, the mussels may be acclimated to river water from Suså and translocated together with male mussels to river locations considered suitable (see section

4.1). The mussels may be investigated for gravidity 2-8 weeks post translocation or during the next reproduction season and used as supplementary brood stock. This method increases the chance of mixing genetic material from Suså mussels, if reproduction in the wild population takes place. However, the translocated mussels should not be used for artificial infestation exclusively as genetic variance in founder individuals is important.

Mussels used for brood collection should be tagged with an individual ID (and a Passive Integrated Transponder, PIT) prior to transfer to their river of origin or to translocation to Suså, as this enables identification and monitoring of the mussels (more information on tagging of mussels is provided in section 4.1.2).

3.1.2 PREREQUISITES AND EQUIPMENT LIST

River temperature should be frequently measured (e.g. using temperature loggers) in April to match timing of brood collection with the start of mussel reproduction. Mussels start glochidia release when river water temperatures reach about 14 ˚C (Schneider, 2017).

A laboratory at which gravid mussels can be maintained for 1-2 weeks is required. It should include a climate chamber where temperatures can be regulated according to stream water temperatures and where constant oxygen supply is available for mussels. A fridge for storing glochidia is required if fish infestation is not carried out directly upon release. Moreover, the laboratory should provide space for working on a microscope binocular and tap water for cleaning of equipment. The latter should be carried out thoroughly before and after entering the river using disinfection solution (e.g. 70 % ethanol), particularly if equipment is moved between rivers and when parasites or invasive species such as *D. polymorpha* are known to occur.

Table 1. Equipment list for mussel brood collection. This list may be incomplete and does not guarantee success.

METHOD	EQUIPMENT
COLLECTION OF GRAVID MUSSELS	Waders; spectrometer; buckets/collection nets for mussels; portable oxygen pumps; mussel tong; lab gloves; camera; transport-containers and stabilization nets; cooling blocks if air temperatures are higher than water temperatures; water barrel; thermometer or temperature logger; field protocols; GPS; mobile phone
MAINTENANCE OF GRAVID MUSSELS IN THE LABORARTORY	Mussel aquaria; oxygen supply; thermometers/data loggers; climate chamber; cleaning equipment; stream water; tab water; multimeter to measure water parameters (e.g. pH, oxygen, temperature)
COLLECTION OF GLOCHIDIA AND MAINTENANCE	50 µm nets; Petri dishes; beakers; pipettes; NaCl; fridge;

3.2 Strategy for artificial infestation of host fish and their release to Suså

Phoxinus phoxinus has been reported to occur at low densities in the Suså system (see section 2.2.1). For strengthening the conservation status of *U. crassus* in the River Suså, *P. phoxinus* is planned to be stocked between the years 2018-2021. A total number of 40300, whereof 40000 are hatchery fish bred from 300 wild fish is supposed to be released to Suså (Table 2). The farmed fish are supposed to be artificially infested with *U. crassus*

SPECIES	YEAR	NO. OF FISH	INFESTATION
P. PHOXINUS	2018	300	No
	2019	10000	Yes
	2020	15000	Yes
	2021	15000	Yes
C. GOBIO	2018	375	No
	2019	375	No
	2020	375	No
	2021	375	No

Table 2. Number of *Phoxinus phoxinus* and *Cottus gobio* individuals planned to be re-introduced in the River Suså between the years 2018-2021.

glochidia prior to their release to Suså. In the following sections, prerequisites for fish infestation and suggestions for its practical implementation are provided, together with general recommendations for a release strategy.

3.2.1 PREREQUISITES FOR FISH INFESTATION

Fish (*P. phoxinus*) provided by a fish hatchery must be acclimated to stream water and local stream water temperatures. To this end, successive water exchange is required, together with constant temperature measurements and investigation of fish behavior. Fish should always be supplied with oxygen. Light stress should be reduced by e.g. covering one side of the container with a dark lid. From a logistic point of view, it is suggested to first transport fish to a place where river water is easily accessible, such as near/at the River Suså, where fish can be acclimated to river water over time (Fig. 13A). This approach reduces stress for the fish as transport takes place in their known chemical habitat. Importantly, the fish delivery must match the timing of glochidia release by gravid mussels. Infestation of fish in the field is convenient, when a glochidia stock solution has been prepared in the laboratory prior to infestation. More information herein is provided in section 3.2.2. Post infestation, the fish can be directly released to the river, hence little, if any fish transport is required afterwards.

If circumstances do not allow for **fish infestation in the field**, this procedure can be carried out in a laboratory or directly at the fish hatchery (Fig. 13B). Here, fish are kept in the water used at the fish hatchery and exposed to glochidia. After fish infestation, the fish are transferred to a fish container supplied with aerated hatchery water free of mussel larvae, in which the fish can be transported to the field. Acclimation to river water and local temperatures is carried out by slowly adding river water to the fish. Fish are released to the river afterwards. Importantly, transport of the infested fish should not be carried out until three days post infestation as accumulated stress to fish should be avoided. Moreover, glochidia encapsulation success on fish gills may increase when fish swimming activity and filtration is moderate during a critical period of three days (Schneider et al., 2017a).

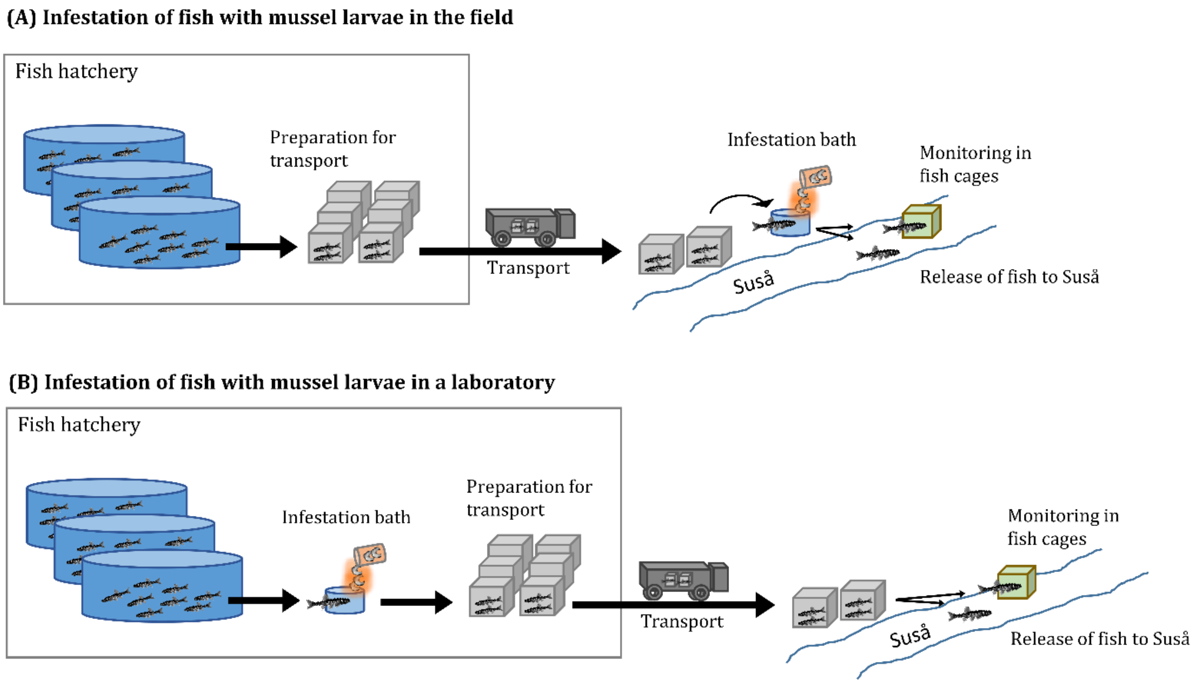

Fig. 13 Sketch of two possible ways to infest fish (*Phoxinus phoxinus*) with mussel larvae of *Unio crassus*.

Feeding of fish may proceed according to the protocol of the fish hatchery. However, feeding of fish prior to release to the river, but after infestation, may increase their survival. We suggest this as released fish may be able to avoid predators to a higher degree when not experiencing trade-offs between foraging behaviour and predator avoidance (Garner et al., 1998).

3.2.2 PREPARATION OF GLOCHIDIA STOCK SOLUTION FOR FISH INFESTATION

Prior to fish infestation, the number of **viable glochidia** collected and pooled from several adult mussels in a so called **stock solution** (volume ~ 0.5-1.0 L) must be quantified in the laboratory. To this end, subsamples (e.g. 6 x 0.5 mL) are drawn (Eppendorf pipettes) from the glochidia stock solution, evenly dispersed by careful agitation. The subsamples are transferred to Petri dishes and glochidia are inspected by means of a microscope binocular. A drop of saturated NaCl solution is carefully added (Fritts et al., 2014). The number of glochidia carrying out valve movements is counted and related to the total number of glochidia in each subsample. By this means, the total number of viable glochidia in the water volume of subsamples can be extrapolated to the water volume of the glochidia stock solution. This procedure is highly recommended to be conducted shortly before fish infestation takes place, to ensure successful glochidia encapsulation on fish. Importantly, the subsamples drawn from the stock solution should not be returned to the glochidia stock solution as NaCl added to subsamples causes closure of all glochidia.

Viable glochidia must be acclimated to the water temperatures at which fish infestation takes place. Transport of the glochidia stock solution to the place of fish infestation is possible when carried out carefully and under temperature control.

3.2.3 FISH INFESTATION

Infestation of fish with mussel larvae is carried out in so-called **infestation baths**. A certain number of fish is transferred to containers fit with a defined volume of water constantly supplied with oxygen. Fish are exposed to glochidia in adding a defined volume drawn from the agitated glochidia stock solution containing the approximate number of glochidia assigned for fish infestation. The infestation bath is agitated for a certain amount of time and fish are released to the river afterwards, or transferred to glochidia-free containers and transported to the river three days post infestation as described above.

The volume drawn from the stock solution for fish infestation depends on the **glochidia concentration**, the **volume of the stock solution** itself, and the desired **glochidia concentration of the infestation bath** (Douda, 2015; Taeubert and Geist, 2017). However, instead of thinking in glochidia concentrations, it is possible to define the number of glochidia for infestation of one fish individual, the desired water volume per fish individual and the number of fish used in an infestation bath (Eybe et al., 2014; Schneider et al., 2017a).

Former infestation experiments from which successful metamorphosis of juvenile *U. crassus* was reported used e.g. a number of 200-1170 glochidia per fish individual in infestation baths of 30 to 45 minutes duration (Eybe et al., 2014; Taeubert et al., 2014; Schneider et al., 2017a; Douda, 2015). For research as for conservation projects, it is recommended to standardize and monitor fish infestation procedures as this enables estimations of glochidia encapsulation and juvenile metamorphosis success, hence evaluation of the mussel re-introduction success. In order to standardize infestation baths, the number of glochidia used per fish individual must be defined, together with the infestation bath volume, which is the glochidia bath concentrations.

If Swedish fish and mussels are used for species re-introduction in Suså, it seems reasonable to follow the infestation procedure described by Schneider et al. (2017a). Here, infestation baths for *P. phoxinus* and *C. gobio* were based on an estimated number of 350 glochidia and 0.3 L water volume per fish individual. This renders an infestation bath concentration of 1050 glochidia L^{-1}. Fish were exposed to glochidia for 30 minutes and the infestation bath was constantly agitated and oxygenated. About 12.2 % of the glochidia used during infestation successfully transformed to juvenile mussels.

3.2.4 INVESTIGATION OF FISH INFESTATION SUCCESS AND JUVENILE OUTCOME

Evaluation of the **infestation success** can be carried out by visual inspection of fish gills from fish ended (benzocaine, > 200 mg L^{-1}) and preserved (95 % ethanol) post infestation (e.g. Taeubert and Geist, 2017; Taeubert et al., 2012a; Douda et al., 2012; Schneider, 2017; Hochwald, 1997, Fig. 14). To this end, fish gills are carefully dissected using scissors and scalpel, and gill arches placed in Petri dished examined for glochidia infestation using microscope binoculars (20-40 x magnification).

Additionally, a water sample can be taken from the infestation bath (agitated water column) to quantify the amount of glochidia that did not attach to the fish. Comparisons to the initial glochidia bath concentration give a rough proxy for the initial infestation rates of fish. However, it needs to be considered, that glochidia that attached to fish skin may fall off the fish prior to successful metamorphosis (Engel and Wächtler, 1989).

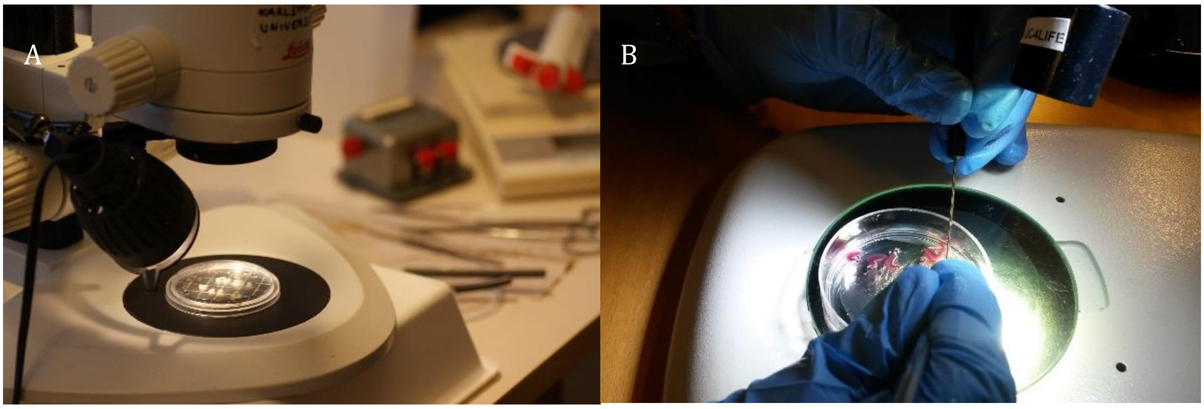

Fig. 14 Dissected fish gill from (A) *Phoxinus phoxinus* and (B) *Cottus gobio* placed under a microscope binocular; pictures taken by Lea D. Schneider ©UC*for*LIFE.

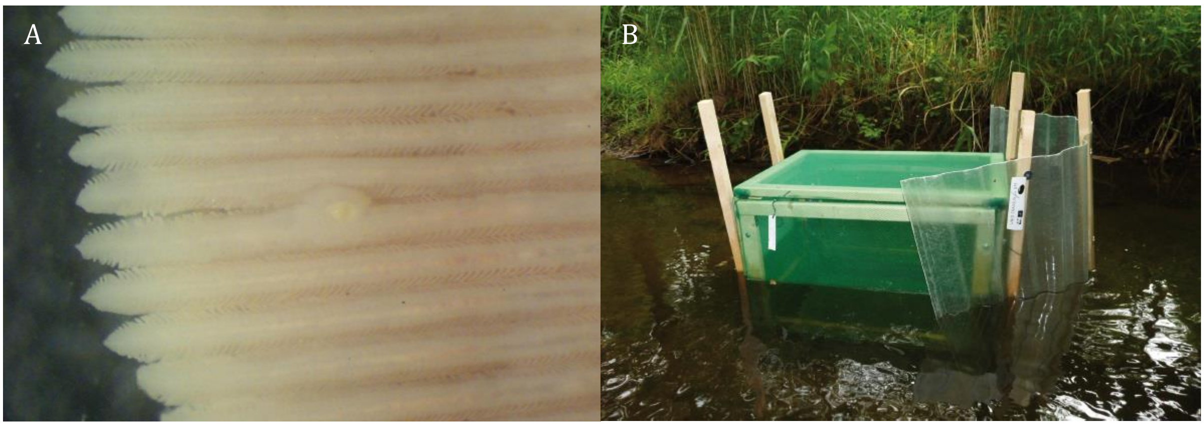

Fig. 15 (A) Fish gill with infestation of one *Unio crassus* glochidium; (B) fish cage placed out in a river and fit with infested *Phoxinus phoxinus*, picture taken by Lea D. Schneider ©UC*for*LIFE.

Monitoring of juvenile metamorphosis success and **fish survival in the field** can be carried out by transferring subsamples of infested fish in cages placed out in the river (Fig. 13 and 15B).

Successive subsampling of fish at e.g. 3 and 16 days post infestation (DPI) gives proxies about the survival of encapsulated glochidia on fish, hence for the potential number of metamorphosing juveniles. However, this method does not provide accurate numbers of metamorphosing juveniles, as such cannot be collected after they fall off the fish. This is due to the small size of juveniles – about 0.2 mm, and potential drift dispersing the juveniles in the river. Hence, the chance of finding the juveniles post hatching from fish in the bottom substratum is very low. However, transport of a subsample of infested fish to a laboratory facility can be carried out to collect and quantify successfully metamorphosed juvenile mussels using mussel hatchery systems. The method is described below in section 4.2.

3.2.5 RECOMMENDATIONS FOR THE RELEASE OF *P. PHOXINUS* TO THE RIVER SUSÅ

From an ecological point of view, the yearly release of 10000-15000 infested *P. phoxinus* to the River Suså should be carried out at **different time points and locations** (an assessment of river locations for re-introduction is provided in section 6). This is important as high numbers of this pelagic fish may affect ecological functions in the river. On the one hand, changes in the food chain can occur when piscivorous fish benefit from introduction of prey items, such as *P. phoxinus*. Hence, parallel stocking of e.g. *S. trutta* should be avoided (Bayerisches Landesamt für Umwelt (LFU), 2013). On the other hand, *P. phoxinus* competes for food with fish, which can have negative consequences for local fish populations, if *P. phoxinus* colonizes freshwaters in high densities such as reported in Norway (Museth et al., 2007; Collin and Fumagalli, 2011). However, drastic invasions of *P. phoxinus* in Norway have mostly been occurring in freshwaters where *S. trutta* was the only local fish species present. In the latest fish investigation in the River Suså, seven and 14 fish species including predators for *P. phoxinus* were detected in the Lower and Upper Suså, respectively (Gørtz and Mouillet, 2017). Most abundant were perch (*Perca fluviatilis*), spined loach (*Cobitis taenia*) pike (*Esox Lucius*) and eel (*Anguilla anguilla*) in the Lower Suså, and perch (*P. fluviatilis*), gudgeon (*Gobio gobio*) and common roach (*Rutilus* rutilus) in the Upper Suså.

The release of 40300 *P. phoxinus* (incl. infested and non-infested fish) to the project river stretches of Suså theoretically results in fish densities of about 0.27 ind. 100 m^{-2}, not taking into account potential fish migration and predators. The future **fish densities** of *P. phoxinus* may therefore even be lower, although depending on the reproduction success of the fish species after release to Suså. These theoretical densities are below fish densities reported from rivers with successful mussel recruitment (Stoeckl, 2016). We therefore suggest that *P. phoxinus*, as shoaling fish species, is released at specific locations together with shoalmates – a method also introducing *U. crassus* juveniles falling off the host fish after parasitism, in a **patchy distribution** along the river parts. This distribution is similar to what is found in nature and may result in so-called mussel beds (Hochwald, 1997).

If re-introduced *U. crassus* are supposed to be found in the River Suså 3-4 years post conservation measure, we recommend to place newly infested fish in **fish cages** set in the river for about four weeks. This is because juvenile mussels falling off the fish post parasitism burry in the sediment below or around the cages and may be found at a later time point when mussels survived and grew larger. This method also enables investigations of the survival of fish in Suså in the absence of predators and of glochidia encapsulation rates on fish (see also section 3.2.4).

3.3 Re-introducion of C. gobio to the River Suså

The aim of *UC LIFE Denmark* is to re-introduce a total number of 1500 *C. gobio* to the River Suså (Table 2). The re-introduction is planned to be conducted over four years (2018-2021), with a number of 375 fish targeted yearly. According to the recommendations of Bollerup (2015), the release of *C. gobio* over a four year period increases the chance of successful re-introduction and survival of the fish. Moreover, successive introduction enables stepwise evaluation of the reintroduction efforts and whether habitat quality is sufficient to continue re-introduction (George et al., 2009). In the following section, recommendations on the practical implementation of the conservation measure are provided.

3.3.1 RECOMMENDATIONS FOR THE PRACTICAL IMPLEMENTATION

Electrofishing is a good means to catch fish along with quantitative estimations of the source populations' density. Importantly, the removal of fish should not pose the wild population at risk. It is therefore suggested to electrofish at several river locations and monitor the fish abundance over the years of fish removal.

The **timing of fish removal** should not meet with the reproduction season of the fish, neither of the reproduction season of affiliated mussel populations, if present in the river. In this way, male fish carrying out parental care are not disturbed, hence the measure does not directly affect reproduction success of *C. gobio*. Moreover, no unknown numbers and species of mussels are introduced to the River Suså via natural glochidia infestation on the fish. As *C. gobio* spawns between February to June, which overlaps with the glochidia release of the freshwater mussels *Unio* spp. and *Anodonta* spp., electrofishing is suggested to be carried out during late fall, when mussel parasitism has passed. Nevertheless, a subsample of fish should be ended (e.g. benzocaine, > 200 mg L^{-1}) and gill-examined (see section 3.2.4) to exclude unforeseen late infestation of glochidia on fish. Fishing in the late fall also ensures that fish condition has improved after spawning in spring, mussel parasitism in spring/summer and good food provision over summer.

From a genetic point of view, fishing should not occur at locations at which **no migration barriers for fish and other aquatic animals** are in close proximity. This is because genetic variation may be low in isolated population (George et al., 2009). Moreover, consideration of the **age class distribution** of re-introduced fish is essential to increase genetic variance and the adaptation potential to local environmental conditions, hence the chance of survival. It is recommended to include multiple age classes (George et al., 2009). Moreover, river locations should be free from potential sources of diseases (IUCN/SSC, 2013).

Caught fish must be acclimated to local river water and temperatures before released to the River Suså (an assessment of river locations for re-introduction is provided in section 6). It is recommended to **acclimate fish** in containers next to the river, where river water is easily accessible. Oxygen should be provided constantly to fish. Depending on the acclimation time, fish containers may be fit with a water pump providing water flow. Experiences with *C. gobio* showed that fungal infections occur and spread fast if no water current is provided to *C. gobio* kept in fish tanks over longer times (Österling and Schneider, 2017). This is due to the sensitive skin of *C. gobio* and their behavior in sitting on top of each other when no hiding structures, such as stones or plastic pipes are provided in fish tanks. However, during fish transport no such structures are recommended, as they may damage and stress the benthic fish. The time for fish acclimation depends on differences in biotic and abiotic water parameters between the river of the source population and the River Suså, and on the time fish must be put under quarantine prior to release to Suså. Measurement of physical-chemical parameters in the rivers (e.g. temperature, oxygen concentrations, conductivity) are therefore important for appropriate acclimation of fish.

PIT tagging of *C. gobio* for manual or automatic monitoring of survival and distribution pattern of the fish post re-introduction has shown to be applicable (Knaepkens et al., 2007), but may pose the fish at risk for fungal infections. **Monitoring of fish presence** may therefore be conducted via electrofishing in Suså, with at least a mid-term and final evaluation conducted during the project. Ideally, fish presence is monitored regularly in the future as part of the national monitoring program (NOVANA).

4. Exit strategy and alternative conservation strategies to reach the project goals

Adaptive management is key to successful conservation of threatened and endangered species (Runge, 2011). It is the awareness of that not all developed conservation strategies proceed according to plan (IUCN/SSC, 2013). Hence, decision-making is an essential part of adaptive management, where monitoring of conservation measures reveal whether such are successful or not. If the conservation objectives are not met, the measures may be discontinued or alternative strategies to reach the conservation objectives are implemented (see also Fig. 10).

Turning points for conservation strategies for *U. crassus* in the River Suså may be ***(1)* a lack of mussel brood** for artificial infestation of host fish, ***(2)* a lack of fish survival** post introduction to the river, or ***(3)* overproportioned success of *P. phoxinus* and *C. gobio*** in the river. If the latter occurs *(3)*, the release of *P. phoxinus* and *C. gobio* may be discontinued and strategies to reduce fish densities implemented. An adaptive strategy herein is proposed below, in section 4.3. Project failure due to a lack of mussel brood from a source population *(1)* may be prevented in using brood from an alternative source population for conservation measures. However, the selection of an alternative source population must be justified and conducted according to the recommendations provided in section 2.1. Alternatively, an additional conservation strategy to re-introduce *U. crassus* in Suså may be applied, e.g. translocation of adult mussels. The method is described in section 4.1. If re-introduced fish do not survive in the River Suså the measure may be paused until further improvements of habitat quality are carried out and environmental conditions meet the species' habitat requirements. Here, it is highly recommend to evaluate the effectiveness and outcome of habitat quality improvements, hence the feasibility for continued fish release. Moreover, it should be evaluated whether fish (*P. phoxinus*) survive better if not infested with mussel larvae prior to their release to Suså. If so, fish and mussels may be re-introduced to Suså using separate strategies. For mussels, one option is the translocation of adult mussels from a source population to the River Suså (see section 4.1). Another option is the rearing (captive breeding) of juvenile mussels at a laboratory and their release to habitat restored river parts in Suså. This conservation strategy is further explained in section 4.2.

4.1 Translocation of adult mussels

Translocation of mussels is a conservation method controversially discussed among conservationists and evolutionary biologists. This is because adult mussels are transferred from one habitat to another to which they are not locally adapted (Olden et al., 2011; Galbraith et al., 2015). Hence, mussels confront changes in environmental conditions and natural selection can lead to reductions of fitness in the new habitat. However, the method is essential when populations must be saved from unfortunate local conditions implying high risk for the mussels (Heinricher and Layzer, 1999; Weeks et al., 2011). Moreover, the method is applied in geographic areas, in which mussel species became extinct and are aimed to be re-introduced in habitat restored freshwaters. Positive results of the method were obtained when mussels were translocated to habitats with stable sediments in which they can burry, and when the measure was conducted during time periods with stable water temperatures (Dunn et al., 1999). Moreover, success of the method on the long run is based on considerations of genetic aspects in founder and source populations related to genetic variation, population size and reproductive potential (see section 1.1.6).

In the River Suså, the translocation of *U. crassus* from a suitable source population seem to be a feasible method to enhance the local population in the Upper Suså. However, in the Lower Suså there is a risk for failure of this conservation approach due to the presence of the zebra mussel *D. polymorpha* (threats to native mussels are described in section 1.1.5). The presence of this invasive species may also reduce the success of other strategies to re-introduce *U. crassus*, such as the release of infested host fish, if the juveniles falling off the fish do not survive due to *D. polymorpha.* It is therefore recommended to conduct a pilot study in the Lower Suså investigating the survival of adult mussels (e.g *Unio tumidus*) translocated from the Upper Suså to the Lower Suså. *Unio tumidus* occurs in high densities in the Upper Suså and is not categorized endangered (Cuttelod et al., 2011; Schneider and Zülsdorff, 2017a). If mussel survival is low during the first summer, as a result of *D. polymorpha* colonization, the translocation of *U. crassus* from a source population to the Lower Suså and the release of infested host fish in the Lower Suså is not recommended. If survival is high, a stepwise approach for mussel translocation is recommended to yearly evaluate the success of this conservation strategy, and to continue or discontinue the measure.

In the next sections, suggestions about the practically implementation and monitoring of the translocation of adult mussels is presented, first for *U. crassus* (sections 4.1.1 and 4.1.2) and for a pilot study on *U. tumidus* afterwards (4.1.3).

4.1.1 PRACTICAL IMPLEMENTATION – *U. CRASSUS*

Careful selection of source populations and river locations from which mussels are removed and translocated to the River Suså is essential prior to the measure. This encompass evaluation of environmental conditions in donor and recipient river, population densities and genetic aspects for source and founder populations. The topic is addressed in detail in section 2.1 of this text.

The **collection of adult *U. crassus*** at a source population should be conducted during the reproduction season of the mussel. This is because mussels emerge to the sediment surface during the warmer months of the year, which facilitates their collection. Moreover, the sex of the mussels can be identified by means of brood inspection in gills (see section 3.1) or by investigating gonad tissue, as a more advanced method (Mioduchowska et al., 2016). It is recommended to translocate mussels with a sex ratio of 1:1, as found in nature. Importantly, it should be considered that mussels not found gravid during visual inspection of gills can also represent females that do not take place in reproduction or already have released their brood. Moreover, translocation of mussels requires consideration of the age structure (Hoftyzer et al., 2008). Although *U. crassus* reproduce life long, fitness decreases can occur with aging (Hochwald, 1997). It is therefore recommended to collect a variety of age classes, however fewer older individuals than younger.

The translocation of gravid mussels is not recommended to be carried out until host fish have been released to the river, hence are available for glochidia released by translocated mussels. However, gravid mussels may be used for collection of brood for artificial host infestation, thus translocated to the River Suså directly afterwards. Alternatively, mussels are collected, sex identified, tagged with an individual number and PIT (see section 4.1.2), placed back to the wild and only translocated to Suså after the reproduction season. In this way, no brood removal takes place in the source population.

Stress during transport and handling of mussels should be kept as low as possible (Dunn et al., 1999). Importantly, sudden **changes in temperatures should be avoided** (Calles, 1980). Transport with constant oxygen provision is recommended and may be carried out in small plastic containers filled with stream water and stabilization material as described above (section 3.1.1). To be able to acclimate mussels to river water from Suså, measurements of physical-chemical parameters (e.g. temperature, pH, conductivity, oxygen) are important to conduct in both the donor and recipient river. Acclimation may take place at the river shores of Suså, where successive water exchange can be carried out. River water from the source population should be collected and disposed as wastewater to avoid introduction of bacteria, parasites or other organisms to the river.

The mussels should be placed in up and downstream direction of the largest native population of *U. crassus* in the Upper Suså. In this way, exchange of genetic material between donor and recipient population is increased. Nevertheless, selection of further locations considered suitable (see section 6) are recommended to increase the chance for success of the mussel translocation to Suså. At each river location, **mussels should be aggregated** to improve the chance for successful reproduction in following reproduction cycles. As a matter of course, possible risks to mussels should be removed as much as possible from the river locations prior to translocation. These include unfavourable habitat quality in the main river and in tributaries, ongoing restoration work and invasive species. Hence, results from a pilot study on *U. tumidus* translocated from the Upper to Lower Suså, which we highly recommended to conducted, should be evaluated prior to translocation of *U. crassus* to the Lower Suså (see section 4.1.3).

4.1.2 MONITORING OF TRANSLOCATED MUSSELS

Translocation of mussels should always be accompanied with **monitoring of survival/mortality** and growth, at least over a two year time period (Dunn et al., 1999). It is also recommended to investigate whether translocated mussels take part in reproduction.

Tagging of mussels using number tags (e.g. bee-tags, numbers laser printed on waterproof paper, and Passive Integrated Transponders, PIT, Fig. 16A) allows for individual identification of mussels and calculation of mortality and growth rates. **Mortality** of marked mussels can be calculated as follows: 'marked shells recovered/(marked life animals + marked shells recovered)x100'. **Growth** can be calculated as the mean increase in shell length (mm) between the time of relocation and monitoring (Dunn et al., 1999).

PIT tagging also facilitates the search for the mussels post translocation, where a portable backpack reader (HDX) with antenna can be used (Fig. 16B). For more information to PIT and HDX see e.g. www.oredonrfid.com. PIT implantation has shown to be less efficient than fixing PIT on mussel shells (Locite superglue and Marine Epoxy), albeit PIT can detach from shells when erosion is high. However, the rejection rate of implanted PIT is high and tags get lost when not grown into the shell of dying individuals (Zając, 2017).The catch of translocated mussels can moreover be facilitated through placing the mussels in grids set up in the river (Dunn et al., 1999).

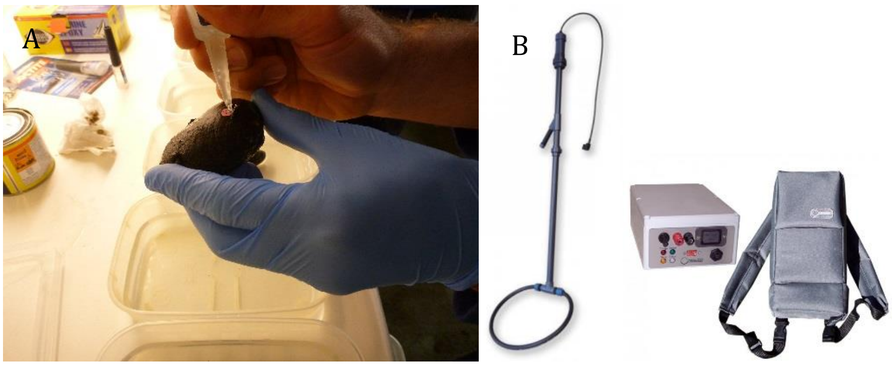

Fig. 16 (A) Tagging of adult mussels (*Unio crassus*) prior to translocation, picture taken by Lea D. Schneider ©UC*for*LIFE; (B) portable HDX backpack reader and pole antenna, from ©OregonRFID.

4.1.3 PRACTICAL IMPLEMENTATION – *U. TUMIDUS*

The pilot study of *U. tumidus* translocation from the Upper to Lower Suså is recommended to be carried out during the reproduction season of *D. polymorpha* in the late spring to summer, however with non-gravid mussels to decrease the chance of introducing *U. tumidus* to the Lower Suså. If introduction of this mussel species to the Lower Suså should absolutely be avoided, the study should not be conducted with living mussels, as the risk of introduction cannot be excluded completely. This is because *U. tumidus* is known to reproduce at multiple times during the reproduction season, similar to *U. crassus* (Hochwald, 2001). Instead, we suggest to conduct a colonization study of *D. polymorpha* with mussel shells only.

Otherwise, living *U. tumidus* are collected at a location in the Upper Suså, where high densities of this species occur (e.g. UC0, see Appendix II.1), and non-gravid mussels are translocated to the Lower Suså, preferably in a grid to facilitate catch post-translocation. Tagging of mussels is recommended additionally. Monitoring of the mussels should be carried out as described for *U. crassus* above, however only during the time period mentioned. The study should end before the mussel starts reproducing in the spring of the next year, if the risk for introduction of this mussel species to the Lower Suså should be lowered (however not excluded completely).

4.2 Rearing of juvenile mussels in an aquaria facility and release to the river

Captive breeding of mussels at an aquaria facility is a common approach for conservation of highly threatened mussel species (Strayer, 2008; Barnhart, 2006). In captivity, the survival of juveniles is usually higher than in nature and enables rearing of high numbers of mussels during relatively short time periods (Hoftyzer et al., 2008). For conservation of *U. crassus* in the River Suså, artificial host infestation, collection and rearing of juvenile mussels at an aquaria facility represents an alternative or additional conservation approach besides the release of infested *P. phoxinus* to the river. In particular, this approach is essential when difficulties with fish farming or survival of the fish in the wild occur. Juvenile mussels can be maintained in the laboratory until host fish are released at a later stage of the project or as long as habitat quality improvements take place in the river. Disadvantages of this conservation strategy for *U. crassus* are linked with high workloads and costs. However, fewer host fish individuals are needed to produce high numbers of juveniles. Moreover, there are at least three different approaches regarding juvenile maintenance in the lab and release to the River Suså. While detailed descriptions of the three approaches are provided in sections 4.2.2 and 4.2.3, a short summary is presented as follows:

Approach 1. Juveniles are collected from artificially infested host fish at a laboratory and are released to the river only few weeks post hatching. No monitoring of juveniles is involved.

Approach 2. Approach 1. including monitoring of juvenile mussels by means of e.g. adjusted Withlock-Vibert Boxes in which juveniles are kept for several months. Survival is investigated regularly.

Approach 3. Approach 1. including monitoring, where juveniles are maintained in plastic boxes at the aquaria facility. The boxes are fit with river water and food, and water exchange is carried out weekly. Survival is investigated at several time points during captivity. Mussels can be kept in the laboratory for several weeks, months or years. Over time, plastic boxes used for maintenance are substituted by other methods according to the 'age' of the juveniles (Eybe et al., 2014). Mussels are released to the river when considered convenient (taking into account temperature regime and habitat conditions).

In the following section, the practical implementation of juvenile collection under laboratory conditions is described and followed by sections addressing approaches 1-3 for maintenance and release of juvenile mussels to the river.

4.2.1 COLLECTION OF JUVENILE MUSSELS

Rearing of juvenile mussels at a laboratory is based on artificial infestation of host fish with mussel larvae, such as described in section 3.2 of this text. From a logistic point of view, farmed fish (*P. phoxinus*) are transported from a fish hatchery to a laboratory where fish are glochidia infested and maintained for juvenile collection. Depending on lab capacities, a number of > 200 host fish are exposed to mussel larvae and transferred to so called mussel hatchery systems (see Eybe et al., 2014, Fig. 17). The systems are water re-circulation systems in which water is one-directionally pumped from one tank to the other. Fish are maintained in the upper tank. In the lower tank, juvenile mussels falling off the fish and transported by the water current are collected in gauze nets. Feeding of fish (frozen chironomids) may be at 4-6 % of the fish body weight every 2-3 day. If fish waste should be reduced during collection of successfully metamorphosed juvenile mussels, fish can be fed at lower rates, which however affects their condition on the long run.

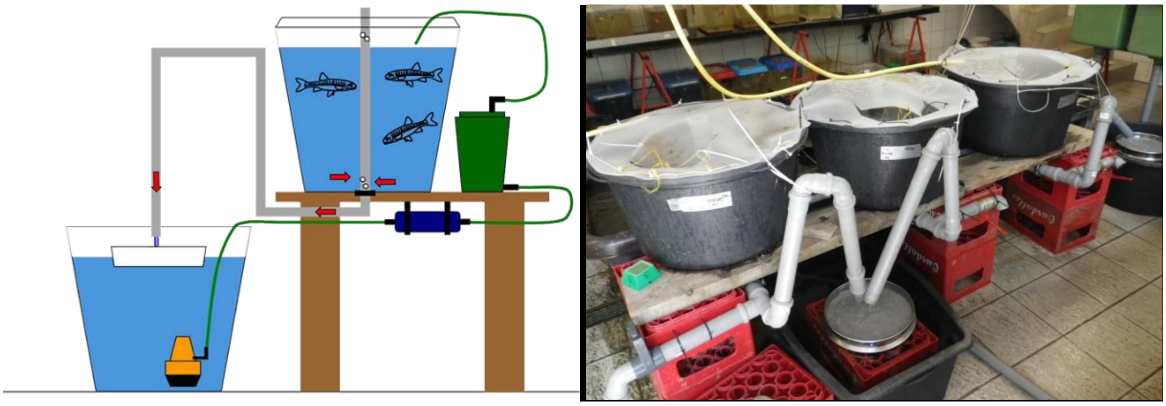

Fig. 17 Mussel hatchery system from Eybe et al. (2014)

The collection of successfully metamorphosed juvenile mussels falling off the host fish may start at the day of infestation, as this ensures that all juveniles are collected. However, glochidia dropping off fish are collected simultaneously, which enables evaluation of host fish suitability. The collection of mussels should be continued until the day post infestation (dpi) when no more juvenile mussels hatch from fish. Gauze nets of < 100 µm mesh size are recommended for collection of mussels. Transfer of glochidia and juveniles to Petri Dishes for quantification can be carried out by means of washing bottles and additional sieves as intermediate steps if needed. Handling of juveniles should be conducted with care, as the mussel shells are weak. Moreover, mussels should not experience sudden changes in temperature and may constantly remain in water they acclimatized. Microscope binoculars (20-40 x magnification) are used for quantification of glochidia and juvenile mussels.

4.2.2 JUVENILE MAINTENANCE IN THE LABORATORY

After collection and quantification of newly hatched juveniles, the mussels are transferred to plastic boxes (0.5-4 L, Fig. 18A) and supplied with stream water and food (detritus, algae, and shellfish diet), according to instructions by Eybe et al. (2014). Eppendorf pipettes (50-1000 µl) are useful tools for the transfer of these little organisms (~ 200 µm). Water exchange and feeding is carried out weekly, together with cleaning of the plastic boxes. Mussels can be maintained in the plastic boxes for about 6-8 months, depending on temperature and development status (Approach 1-2). However, mussels should latest be transferred to sand-aquaria when gills develop and juveniles switch from pedal feeding to filter feeding (Approach 3, Fig. 18B). At a size of > 6 mm, mussel can be transferred to gravel-baskets fit in flow through systems (Fig. 18C). Detailed information on the methodology of these three rearing systems used for *U. crassus* is provided by Eybe et al. (2014). As these methods have proven successful for *U. crassus*, no further methods currently used in mussel propagation, particularly in North America, are addressed in this text. However, information herein can be obtained from a comprehensive book recently published by Patterson et al. (2018).

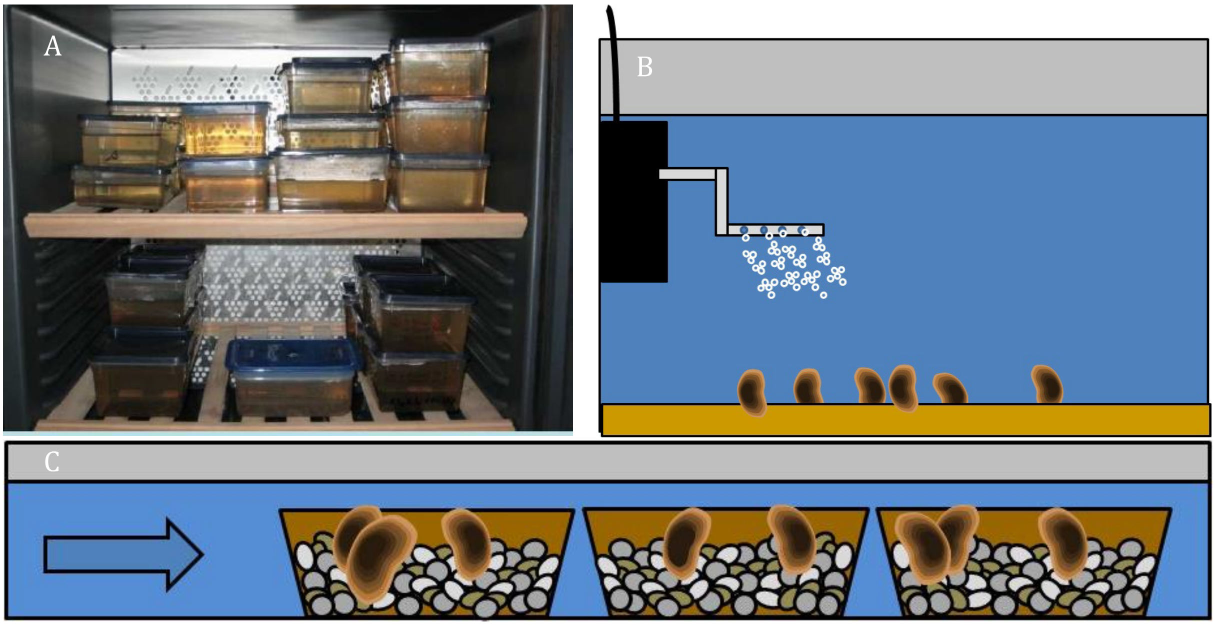

Fig. 18 Different methods (A, detritus boxes; B, sand-aquaria; C, gravel baskets) for maintenance of juvenile mussels in the laboratory, from Eybe et al. (2014).

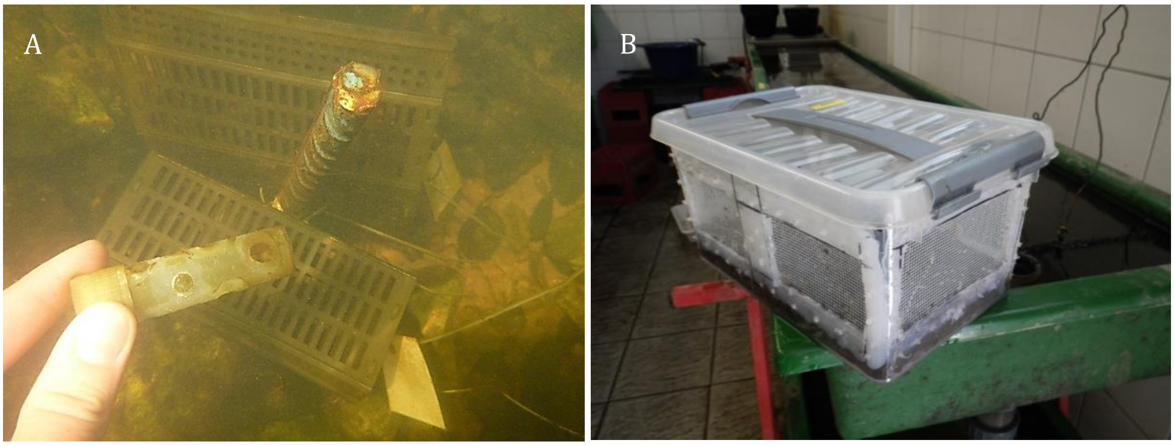

Fig. 19 (A) Adjusted Withlock-Vibert boxes, (B) picture taken by Lea. D. Schneider; gravel baskets with mussels that are placed out in the river, from Eybe et al. (2014).

4.2.3 RELEASE OF JUVENILE MUSSELS TO THE RIVER

As introduced above, there are different approaches for the release of juvenile mussels to the field. The first approach (Approach 1) is simple and least costly, although the juvenile outcome is difficult to predict. However, juveniles can be quantified in the laboratory prior to release. Adult mussels can be detected in the river 4-5 years later. The release of juvenile mussels can be carried out by means of tubes (diameter 4-12 cm) placed in the river bottom but ranging over the water level. Juveniles are transferred to the tubes and flushed down to the water inside the tubes. After some time in which mussels burry in the river substratum, the tube is carefully removed. By this means, the distribution of the mussel is somewhat limited compared to releasing the mussels in the free flowing water, where catch is more difficult.

Generally, juvenile mussels newly hatched from fish and released to the river have a lower chance of survival than juveniles bred in captivity until grown larger (McMurray and Roe, 2017). Monitoring (Approach 2) facilitates investigation of the survival of young juveniles in the field and can be carried out by means of adjusted Whitlock-Vibert Boxes, in which mussels are placed in tubes inside the boxes (www.ucforlife.se; Fig. 19A). Before winter, the juveniles are however freed from the boxes and released to the river, because maintenance and survival in the free flowing water cannot be guaranteed when temperatures decrease.

At a later stage of juvenile development, when mussels grew larger (> 2 cm) in the laboratory, mussels can be transferred to gravel baskets placed out in the river (Approach 3, Fig. 19B). A more detailed description is provided by Eybe et al. (2014).

4.3 Removal of fish

In the unlikely event of overproportioned success of *P. phoxinus* and *C. gobio* in the River Suså (see section 3.2.5), the release of host fish may be discontinued and strategies to reduce fish densities implemented. An adaptive strategy herein is the removal of fish by means of fishing (e.g. minnow traps, electrofishing). Top-down control as biomanipulation approach is not recommended as the release of piscivore fish (e.g. *E. lucius*, *S. trutta*), generally aiming to reduce cyprinids and hereby phytoplankton abundance (incl. cyanobacteria), greatly affects the food chain of a freshwater and is not target-specific. Moreover, top-down control requires careful planning and monitoring (Baer et al., 2007).

Electrofishing is a good means to catch high numbers of fish at low cost. However, this method is originally intended to be used for fish monitoring and not for fish removal. Nevertheless, electrofishing is target-specific as catches not representing *P. phoxinus* or *C. gobio* can be released to the river unharmed. Moreover, electrofishing allows for quantitative estimates of the fish composition and abundance, which is required at first to evaluate the success of *P. phoxinus* and *C. gobio* in the River Suså post release. In general, a midterm and a final fish monitoring is recommended post fish release to Suså, if not conducted more often. Additionally, monitoring may include questioning of local fishermen about observed changes in fish composition and abundance.

Threshold fish densities in Suså representing turning points of adaptive management are dependent on the impact of released fish on the river system. It is recommended to consult experts on ecosystem functioning in relation to fish abundances, if mass occurrences of the targeted fish species are noticed post release.

5. Cost-benefit analysis of conservation strategies

Adaptive management of conservation of highly threatened species should include feasibility assessment of conservation strategies based on 'a balance of the conservation benefits against the costs and risks', including alternative conservation strategies to reach the original conservation aims (IUCN/SSC, 2013). In the following sections, a cost-benefit analysis is presented for each of the four major conservation strategies presented in this text, which are the release of infested host fish (*P. phoxinus*) with *U. crassus* glochidia (section 5.1), the translocation of adult mussels to the River Suså (section 5.2), captive breeding of *U. crassus* with successive release of juvenile mussels to the river (section 5.3), and the re-introduction of *C. gobio* to the River Suså (section 5.4).

5.1 Release of fish (P. phoxinus) artificially infested with mussel larvae

The release of artificially infested *P. phoxinus* to the River Suså is a good way to re-introduce *U. crassus* and its host fish simultaneously. This conservation strategy is relatively simple and cost efficient compared to captive breeding of mussels, particularly when survival of fish and juvenile mussels falling off the fish post release is high.

Habitat quality in the river is key factor for survival of fish and mussels. Hence, careful removal of threats that have been causing the decline and extinction of the species is essential prior to species re-introduction. Moreover, improvement of habitat quality according to the needs of the target species (see section 1) increases the chance for survival and reproduction in the end. Beneficial with releasing pelagic fish species, such as *P. phoxinus*, is their migration potential enabling the fish to actively choose their preferred habitat in the river and to switch between microhabitats for e.g. spawning, feeding and hiding (see section 1.2). This ability increases their chance of survival post release. Moreover, it is likely that mussels that successfully passed parasitism on fish and survived until the adult life stage can be found at the preferred habitats of the fish at approximately 3-6 years post release of the infested host fish.

However, fish can also move away from river stretches targeted in the conservation project, which renders difficult quantification of fish and mussel survival locally, hence the outcome of the conservation strategy. An easy way to limit the **distribution of juvenile mussels** via host fish migration is the maintenance of infested fish in cages placed out the river for the time of mussel parasitism. The measure is relatively low in costs and only temporary work intense. Moreover, it excludes predation on the encaged fish and enables investigation of fish survival in this condition, hence adaptive management.

Adaptive management is important from an ecological point of view, but also regarding costs. Farming of fish (n ~ 40000) represents the most cost intense part of the conservation strategy discussed here. Hence, reduced fish survival post release linked with e.g. adverse habitat conditions, negatively affects the cost-efficiency of the conservation strategy, but can be reduced if adaptive management is applied. Here, monitoring of fish abundances poses additional costs, but enables decisions on e.g. the need of additional habitat improvement prior to continued release of fish, the exclusion of river locations at which fish are released, and the discontinuation of fish release.

Success of the conservation strategy further requires careful planning and coordination of fish infestation. This includes spatial and temporal matching of brood collection from gravid mussels with the delivery of farmed fish and temporal adaptation of actions to environmental conditions, e.g. the timing of brood release by mussels. Beneficial for the practical implementation of fish infestation is the reproduction mode of *U. crassus*. In a mussel population of different age classes, brood fertilization and glochidia release occur asynchronous and at multiple occasions during the mussel reproduction season, which is in contrast to other mussel species such as *Margaritifera margaritifera*. Hence, it is likely that gravid *U. crassus* can be found during the entire mussel reproduction season. With careful selection of a source population, it is likely that successful fish infestation and release of infested *P. phoxinus* to the River Suså can take place during each of the planned project years (2019-2021).

To evaluate whether the aims of *UC LIFE Denmark* - to re-establish a mussel population of 0.1-0.2 mussels m^{-2} at the project sites, which corresponds to about 10000 mussels, can be met by releasing 40000 infested *P. phoxinus*, a highly simplified ***hypothetical model*** is presented as follows. The model is based on parameters that can be approximated (e.g. the number of fish available for infestation, the number of glochidia used during fish infestation and the potential metamorphosis success of juvenile mussels), and on parameter that are unpredictable, but affect the outcome of adult mussels in Suså (e.g. glochidia viability, predation risk for *P. phoxinus* in the River Suså, fish mortality related to water quality, habitat conditions affecting survival of juvenile mussels and risk for predation of juvenile mussels in the wild). It assumes that 40000 fish individuals are infested with a number of 350 glochidia per fish individual, and that glochidia successfully metamorphose to juveniles at about 12 % (Schneider et al., 2017a). To account for unpredictable parameters potentially reducing the outcome of mussels in the River Suså, a risk factor of 100 is assigned randomly. In this way, the hypothetical model can be run in a more holistic way somewhat accounting for unknown risks. Finally, the result of the hypothetical model (equation: 40000 fish x 350 glochidia fish^{-1} x 0.012 metamorphosis success x 100^{-1} risk factor) implies that ~17000 mussels may fall off the host fish, burry in the river bottom of Suså and emerge as adults. This hypothetical value is higher than the project goals'. Nevertheless, this hypothetical model elucidates how difficult it is to predict the outcome of the conservation strategy. The true outcome may be both, higher or lower than predicted. Concluding it can be said that the overall conservation outcome can only be evaluated 3-6 years post conservation measure, when juvenile mussels reached a size visibly for the naked eye during sieving of sediments or when adult mussels emerge to the sediment surface.

5.2 Mussel translocation

Translocation of adult mussels represents the simplest conservation approach presented in this management plan to re-introduce *U. crassus* in the River Suså. This measure is regarded as alternative conservation measure **feasible** to reach the project goals. The costs for collection of mussels at a source population, acclimation of mussels to local river water and the release of mussels to the river are low. This is because little equipment and working effort is required. Hence, compared to other conservation measures, such as the release of artificially infested host fish to the river, and captive breeding of mussels in a laboratory facility, the translocation of mussels is highly cost efficient.

Mussel monitoring, which should be part of the conservation approach, allows evaluation of mussel fitness (survival and reproduction potential), and helps deciding whether the conservation measure should be continued or discontinued. Costs may be related to investigation of mussel survival, growth and reproduction, for which individual tagging of mussels prior to translocation is recommended. However, it is likely that costs related to monitoring of translocated mussels are lower compared to monitoring measures of other conservation strategies.

The **translocation success** depends on the fitness of the mussels in their new habitat, which is affected by local environmental conditions and the adaptation potential of mussels to such. Generally, suitable habitat conditions increase survival and fitness. However, both can be reduced due to moving away of mussels from their native river systems to which they are adapted through evolutionary history. Hence, success of the conservation strategy is based on careful consideration of ecological and genetic aspects for source and founder population. This should encompass comparison of environmental parameters between donor and recipient river, population genetic analyses for selection of the most suitable source population, and the size of the founder population which should allow for successful reproduction. If survival and fitness of mussels is high during the first years post translocation, the measure can be regarded as sustainable with a low cost-benefit ratio. Actually, the translocation of adult mussels is regarded as one of the most effective techniques in re-introducing mussels (McMurray and Roe, 2017).

5.3 Captive breeding of U. crassus and successive release to the river

Captive breeding is a common and highly quantitative conservation approach for re-introduction of threatened mussels to habitat restored freshwaters. The approach bridges the natural difficulty for mussel larvae to encounter suitable host fish and for newly transformed juvenile mussels to reach habitats of high quality post parasitism. Hence, captive breeding allows for production of high numbers of juvenile mussels by means of relative low numbers of host fish artificially infested under controlled laboratory conditions. The reared juveniles can be released at a defined point in time. The older the juveniles are at release to the wild, the higher is their chance for survival (McMurray and Roe, 2017).

In general, this approach is regarded as the safest, but most time and cost intense conservation strategy compared with other approaches, such as the release of artificially infested host fish and mussel translocation. Costs are related to maintenance of an aquaria facility and equipment, labour and expertise required to collect and rear the juveniles all along. However, if the conservation strategy encompasses the release of relatively newly transformed juveniles, time and effort can be reduced as laboratory effort is 2-4 months.

The collection of juvenile mussels from artificially infested fish at an aquaria facility allows for quantitative assessment of juvenile metamorphosis success and survival in the lab. Combined approaches to quantify juvenile survival in the field moreover provide proxies for the success of the conservation effort in nature. Furthermore, artificial infestation of fish under laboratory conditions enables tests of host fish compatibility. Hence, this approach is unique to identify physiological host fish. In the River Suså, there are at least three fish species that are potential hosts for *U. crassus*, namely *P. fluviatilis*, *L. lota* and *R. rutilus*. Regarding the current budget of *UC LIFE Denmark*, host fish testing may however not be feasible.

Overall, the feasibility of captive breeding of mussels during the time frame of *UC LIFE Denmark* is evaluated high, if an aquaria facility including all equipment required is available. Most of the equipment is custom-made. So far, no such facility exists in Denmark. Nevertheless, any aquaria facility can be adjusted to captive breeding, if a budget, time, manual skills and expertise is given. Alternatively, existing facilities at which mussels have been reared previously can be used, although they are not in close proximity. The closest laboratory may be the laboratory at Hemmerstorps Mölla, near Veberöd in southern Sweden. Concluding, feasibility of this conservation approach highly depends on the project budget, but also on the time point of project turning points when alternative conservation methods, such as captive breeding are required to reach the project goals.

5.4 Re-introduction of C. gobio to the River Suså.

Since the 1960th, *C. gobio* is extinct in Denmark. Conservation of this fish species in the River Suså, known to represent the former distribution area of *C. gobio*, is therefore based on a fish translocation from a source population. From a biological point of view a source population from southern Sweden is considered feasible. Electrofishing, acclimation of fish to Suså and release of the fish to the river represent low-cost methods. The original project plan to infest the fish with mussel larvae is not recommended, as the chance for successful re-introduction of this fish species valuable for Denmark should be maximized. Hence, no additional cost accrue. The practical implementation can start in 2018, after approval of Swedish and Danish authorities.

5.5 Conclusions

The translocation of adult mussels is the most cost effective way to introduce *U. crassus* in the River Suså. Captive breeding represents the most quantitative, save and expensive conservation approach. However, compared to the release of ~40000 *P. phoxinus* infested with *U. crassus* glochidia, these two conservation approaches do not focus on host fish re-introduction to Suså, albeit essential for the future success of introduced mussel populations. Hence, the release of infested *P. phoxinus* represents the conservation strategy most sophisticated from a biological point of view, and may also hold the lowest cost-benefit ratio. The latter derives from a relative high potential for success of the conservation strategy, mainly due to the high numbers of infested fish planned to be release to Suså, and relatively low costs. However, using mussel translocation as parallel strategy to re-introduce *U. crassus* in Suså represents a low-cost back-up for conservation success that can be combined with the release of artificially infested fish in the practical implementation, e.g. during brood collection.

The re-introduction of *C. gobio* is an additional benefit for future mussel populations of *U. crassus* and represents a historically founded conservation approach for this fish species in Denmark.

6. Assessment of locations for species enhancement and re-introduction

The selection of locations for species re-introduction should be based on the habitat quality and possible risks that may reduce the success of the conservation measure (IUCN/SSC, 2013). Here, evaluation of habitat quality should address biotic and abiotic parameters, and should take into account the former species-distribution. In this section, an overall assessment of river stretches of the Lower and Upper Suså is conducted. In sections 6.1, 6.2 and 6.3, this information is used to suggest target species-specific and life stage dependent (juvenile and adult mussels) locations for re-introduction.

Water quality parameters extracted from the national monitoring program (NOVANA) show that a general trend towards lower values of nitrogen and phosphorous has been occurring in the Upper and Lower Suså since the 1980th, when the monitoring started (Appendix I). During the years 2000-2017, a reduction of the biological and the chemical oxygen demand (BOD_5 and COD) occurred. However, the BOD_5 levels in the Lower Suså were at general higher levels than in the the Upper Suså (Table 3). Hence, the BOD_5 level of the Upper Suså reflect what is recommended for salmonid rivers ($BOD_5 \leq 3$ mg L^{-1}), and the BOD_5 levels of the Lower Suså are at levels recommended for cyprinid rivers ($BOD_5 \leq 6$ mg L^{-1}, Bayerisches Landesamt für Umwelt (LFU), 2013). The nitrate nitrogen levels in Suså ranged around 2.5 mg L^{-1}, however were almost twofold higher in the tributary Ringsted Å than in the River Suså (Table 3). The pH values ranged around 8-9 and dropped seldom below 7 (not shown in Table 3).

Table 3. Overview of chemical parameters measured in the Lower and Upper Suså (incl. tributaries) between the years 2000-2017; extracted from NOVANA. Data represent average values with ± standard deviation. TN, total nitrogen; TP, total phosphorous; BOD5, biochemical oxygen demand in 5 days; COD, chemical oxygen demand; Ortho-P, ortho-phosphate; NH3 NH4-N, ammoniac ammonium nitrogen; NO2 NO3-N, nitrite nitrate nitrogen; NO3-N, nitrate nitrogen; NO2-N, nitrite nitrogen; NA, data not available.

River part	Location	TN	TP	BOD5	COD	Conductivity	Ortho-P	NH3 NH4-N	NO2 NO3-N	NO3-N	NO2-N
	Name	mg/L	mg/L	mg/L	mg/L	mS/m	mg/L	mg/L	mg/L	mg/L	mg/L
Lower Suså	S.f.Holløse Mølle	3.08 ± 1.53	0.12 ± 0.06	2.26 ± 1.98	25.77 ± 9.18	58.23 ± 5.83	0.08 ± 0.05	0.08 ± 0.07	2.39 ± 1.60	2.42 ± 1.64	0.08 ± NA
	N.f. Holløse Mølle	NA	NA	4.40 ± 2.70	NA	NA	NA	NA	NA	NA	NA
Upper Suså	Næsby Bro	4.12 ± 2.13	0.14 ± 0.7	1.35 ± 0.62	NA	71.33 ± 3.09	0.08 ± 0.04	0.09 ± 0.1	3.25 ± 3.06	NA	NA
	Almtofte	3.7 ± NA	0.10 ± NA	1.63 ± 0.64	NA	72.20 ± 5.88	0.06 ± 0.05	0.05 ± 0.09	2.28 ± 1.01	2.60 ± 1.38	0.08 ± 0.02
	Vetterlev Bro	5.30 ± 2.85	0.14 ± 0.06	NA	NA	NA	0.06 ± 0.03	0.14 ± 0.21	4.60 ± 2.67	NA	NA
	Vetterslev, Ny Bro	4.68 ± 2.31	0.11 ± 0.04	NA	NA	NA	0.06 ± 0.03	0.08 ± 0.04	3.86 ± 2.28	NA	NA
Tributary of Upper Suså	Vrangtrup, Ringsted Å	3.93 ± 2.04	0.13 ± 0.06	1.37 ± 0.05	NA	NA	0.08 ± 0.05	0.13 ± 0.31	2.82 ± 1.82	4.14 ± NA	NA
	Vasebækken	NA	NA	NA	NA	NA	0.07 ± 0.01	0.09 ± 0.09	5.66 ± 0.24	NA	NA
	Stavnebæk	NA	NA	1.12 ± 0.28	NA	NA	NA	NA	NA	NA	NA

As far as the monitoring data can be compared with data reported from other *U. crassus* rivers in Europe (see section 1), the chemical water parameters in Suså fall in the range of ecological plasticity of the mussel and its host fish species (*P. phoxinus* and *C. gobio*). However, based on the water quality data available, a more precise evaluation of habitat suitability for *U. crassus* is difficult. First, few measurements were conducted recently, and secondly, few if any data is available for nitrate nitrogen, nitrite nitrogen, ammonium nitrogen and phosphate phosphorus in both the free flowing water and the interstitial of the river. Measurements planned in 2018-2019 at locations in the Upper and Lower Suså may help evaluating the chemical habitat quality in Suså for the target species. However, in this text, we base our suggestions on suitable river locations on physical and biological parameters, such as provided by an investigation of the ecological status in Suså, where information on physical habitat quality and species composition of benthic fauna is provided (Birkolm Hansen and Wiberg-Larsen, 2017). Moreover, results from an *U. crassus* inventory (Schneider and Zülsdorff, 2017a) and from an investigation of fish species conducted in the River Suså (Gørtz and Mouillet, 2017) are considered.

The investigation of the ***ecological status*** carried out in the River Suså in 2017 showed that the Upper Suså holds an overall higher Danish Physical Index (DFI, Dansk Fysisk Indeks) than the Lower Suså. This is mainly based on that eight of ten locations investigated in the Lower Suså are impounded due to the dam at Holløse Mølle (Birkolm Hansen and Wiberg-Larsen, 2017). The benthic fauna composition investigated and evaluated by means of the Danish Stream Fauna Index (DVFI, Dansk Vandløbs Fauna Indeks) reflects the impoundment as species adapted to slow moving waters/lakes are strongly represented above the dam, including high numbers of the invasive zebra mussel (*D. polymorpha*). In contrast, the species composition in the Upper Suså and downstream of the dam at Holløse Mølle, reflects more stream dwelling species. No *D. polymorpha* was recorded in the Upper Suså, however the species also occurs below the dam of Holløse Mølle in the Lower Suså (Schneider and Zülsdorff, 2017a). Despite the DFI categorization of a good/moderate to high ecological status in the Upper Suså (except of location N f Klintebjerggård holding a moderate DFI, Table 6), relatively few clear water indicator species were found in all of Suså. Moreover, presence of green algae was noted at several locations in both the Upper and Lower Suså (Birkolm Hansen and Wiberg-Larsen, 2017).

Regarding the ecological status of Suså, there are at least three locations the ***Upper Suså*****,** that represent overall suitable river stretches for species re-introduction, particularly if small habitat improvements such as placing out woody debris, large boulders and gravel for fish spawning, are carried out (see more in section 7). These river stretches are located near the bridge at Assendrup, at Eskildstrup Møllebro, and north of Aversi (Fig. 20). The location near Assendrup is important from both an ecological and a genetic point of view, as the river stretch lies downstream of the currently known *U. crassus* population (n=6). More information of beneficial habitat conditions of these river locations, but also disadvantages and risks for species re-introduction are provided in Table 4. Likewise, locations considered less suitable for species re-introduction compared to the three locations mentioned above are presented in Table 6. However, these locations may represent suitable habitats for the target species, if habitat improvements are carried out (see more in section 7).

The river restoration planned for the ***Lower Suså*** will bring along beneficial changes in hydrology, erosion and sediment composition transforming the impounded area into a flowing river. However, unstable sediments, high levels of erosion and nutrient loads are likely to occur in the first years post restoration until the new ecosystem system stabilized, taking > 1-2 years. This includes changes in species composition of flora and fauna. For instance, an increase of *D. polymorpha* may occur below the dam and may last for several years until mussel abundance

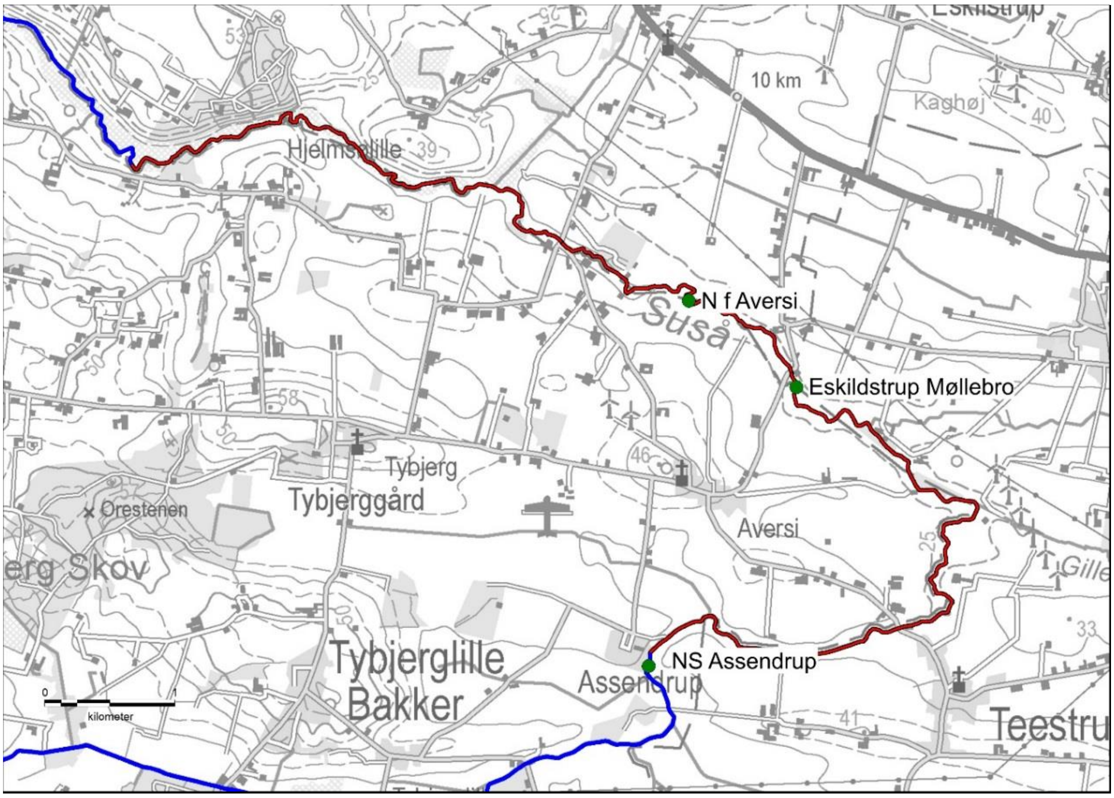

Fig. 20 Map of the Upper Suså indicating river locations considered most suitable for species re-introduction. The map was kindly provided by Næstved Municipality.

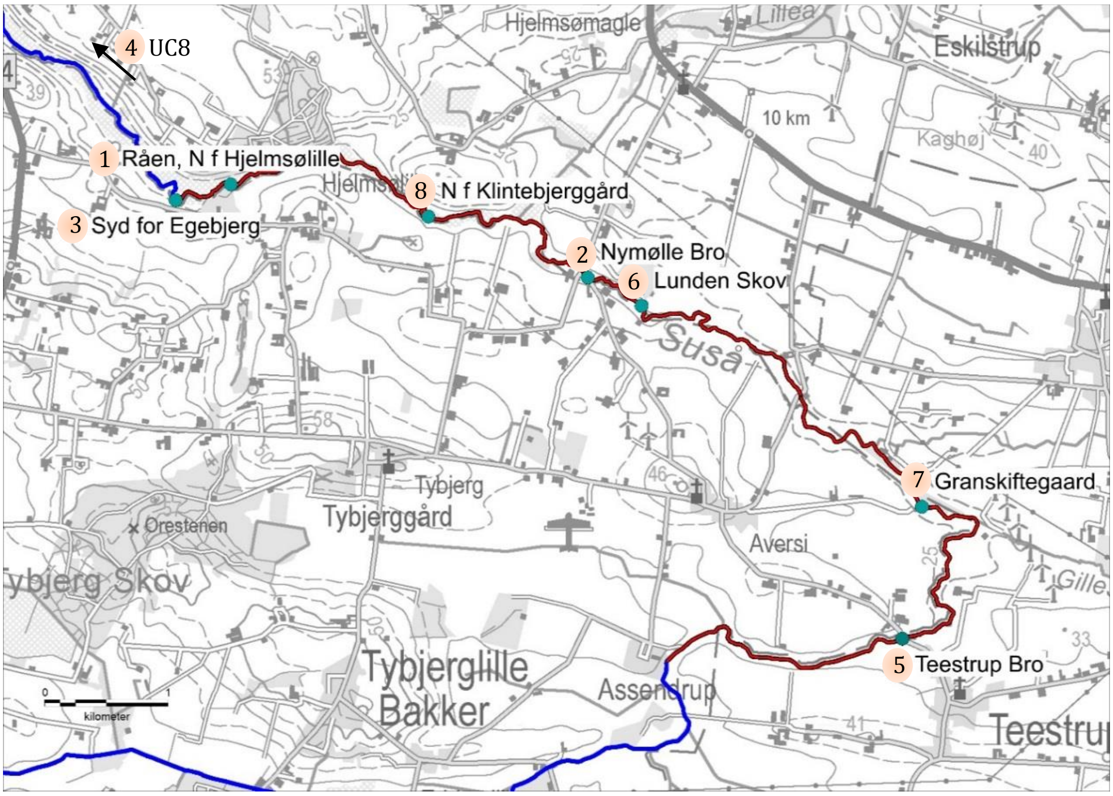

Fig. 21 Map of the Upper Suså showing river locations considered less suitable for species re-introduction prior to habitat restoration, however considered more suitable post restoration. A ranking is made for the locations depending on their evaluated suitability for species introduction. RANK 1 represents the location evaluated most suitable. The map was kindly provided by Næstved Municipality.

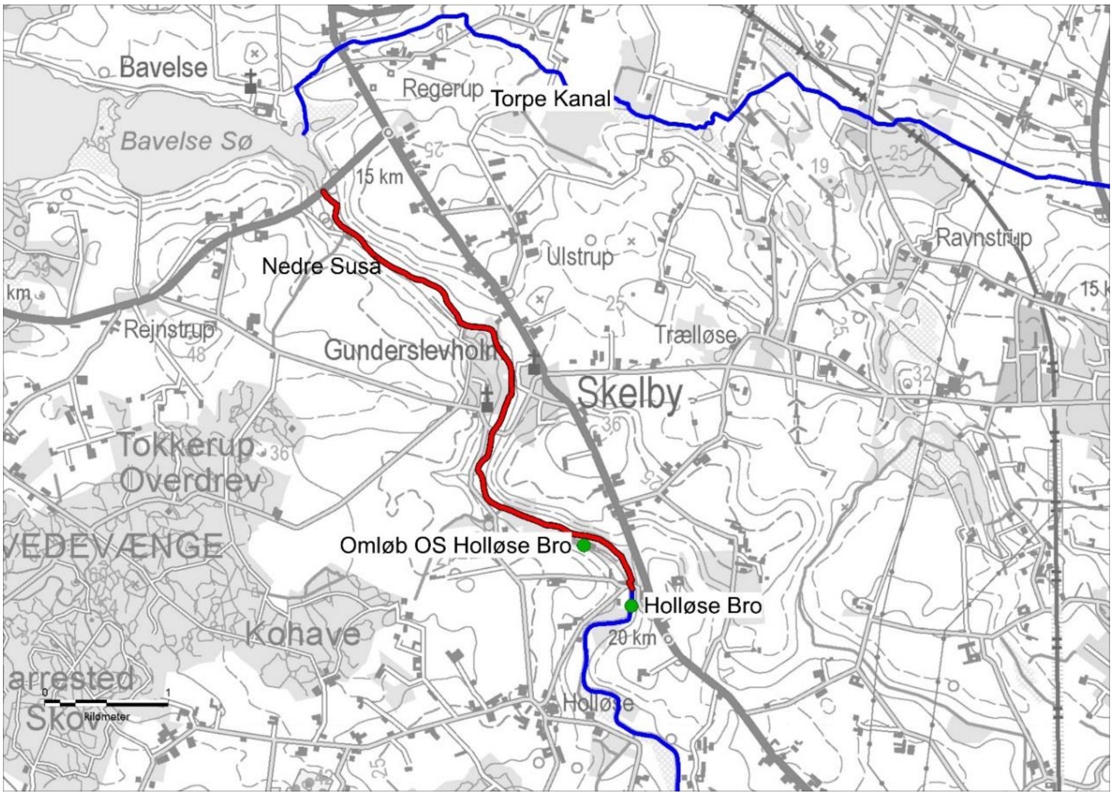

Fig. 22 Map of the Lower Suså indicating river locations considered suitable for species re-introduction post habitat restoration. The map was kindly provided by Næstved Municipality.

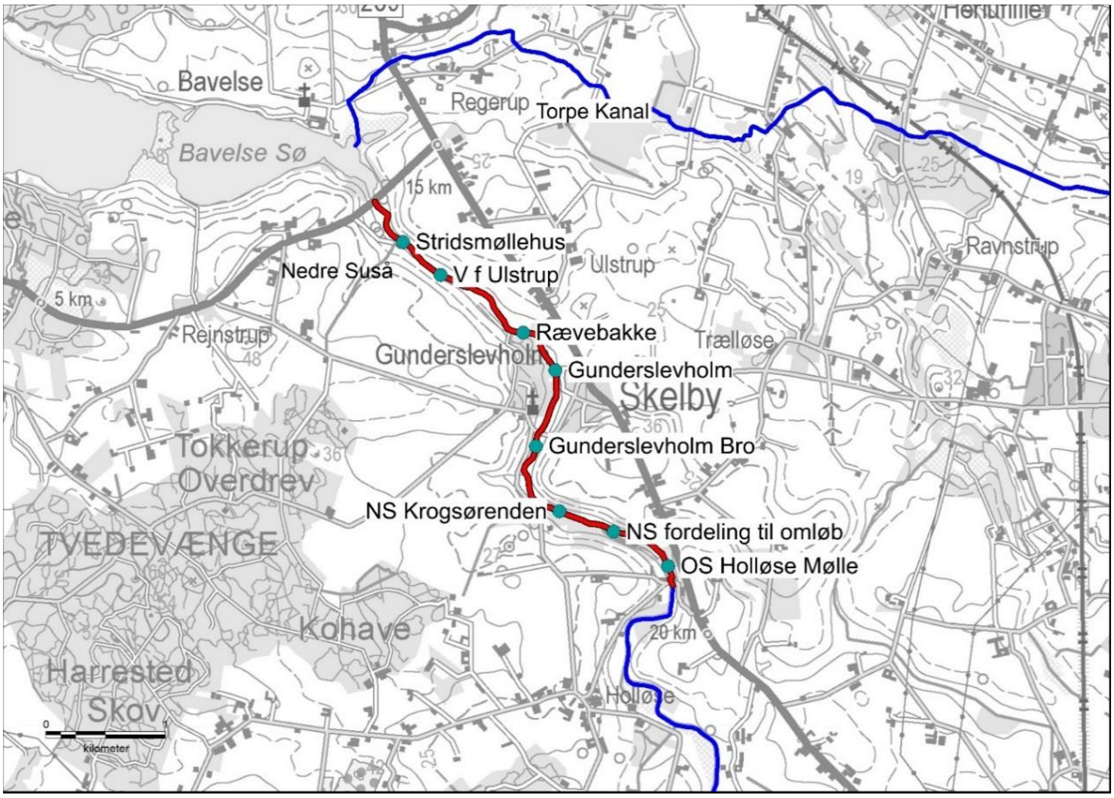

Fig. 23 Map of the Lower Suså showing river locations not considered suitable for species re-introduction before any river restoration has been carried out. However, the project stretch (indicated in red) is assumed to represent suitable habitat for *U. crassus* and its host fish after the restoration, particularly between Holløse Mølle and Skelby. The map was kindly provided by Næstved Municipality.

Table 4. Summary of potential locations for species re-introduction in the Upper Suså. The names of the river locations correspond to locations shown in Fig. 20 and in Appendix II. Information on the ecological status of Suså (DFI, Danish Physical Index ; DVFI, Danish Stream Fauna Index) is extracted from Birkolm Hansen and Wiberg-Larsen (2017). Information about fish species in the river is extracted from Gørtz and Mouillet (2017). Other information derives from Schneider and Zülsdorff (2017a) and personal communication with experts.

Location	Benefits	Disadvantages and Risks
NS Assendrup (Between UC0-UC1; Ø1)	- DFI: good/moderate - DVFI: 4 (10 pos., 3 neg.); 45 species/groups; clear water indicator species present - Downstream of the last remaining mussel population - Trees and the bridge provide shadow - Tree roots in the water - Intermediate heterogeneity of sediment (granules, pebbles, cobbles, some mud) - Submerged vegetation (hiding places for fish) - Upstream of tributary no green algae - Fewer *Unio tumidus* compared to UC0 - Food for fish, e.g. *Gammarus pulex* - Potential host fish species for *U. crassus*: *P. fluviatilis*, *R. rutilus*	- Low variation in flow velocity - No backwater - Steep river edges (no retention zones) - Unknown fish spawning ground quality
Eskildstrup Møllebro (UC4, Ø5)	- DFI: good/moderate - DVFI: 4 (9 pos., 3 neg.); 38 species/groups - Buffer zone to river at the right side of the river looking in upstream direction - Single trees and the bridge provide shadow - Three roots and macrophytes provide shelter for fish - Food for fish, e.g. *Gammarus pulex* - Potential host fish species for *U. crassus*: *P. fluviatilis*, *R. rutilus*	- Low flow variation - Steep river edges (few retention zones) - Unknown fish spawning ground quality - Relative low sediment heterogeneity - Green algae
N f Aversi (UC5, Ø6)	- DFI: high - DVFI: 4 (9 pos., 4 neg.); 34 species/groups; clear water indicator species present - Buffer zones to fields - The river goes in meanders and provides retention zones, as well as shallow and deeper zones - Shadow provided by trees and riparian vegetation hanging into the river - Intermediate sediment heterogeneity - Recruitment of *U. tumidus* assumed - Possible spawning ground for fish - Food for fish, e.g. *Gammarus pulex* - Potential host fish species for *U. crassus*: *P. fluviatilis*, *R. rutilus, L. lota, Pungitius pungitius*	- Limited access to the river from land - Few macrophytes

decreases according to a typical species invasion curve. The potential increase of *D. polymorpha* can derive from the new fauna passage allowing mussel larvae to drift downstream, and from a nutrient increase linked with changes in hydrology and excavating. However, settlement of *D. polymorpha* larvae greatly depends on the flow. If flow velocity is high, the species' abundance may decrease in the project area instead. However, predictions on colonization patterns of *D. polymorpha* are difficult to make and may be wrong. An investigation encompassing densities of *D. polymorpha* is recommended one year after the river restoration to evaluate whether habitat quality in the river stretches suggested in the present management plan is still suitable for species re-introduction. In any case, river locations at which low densities of *D. polymorpha* occur are recommended for species re-introduction. Moreover, the release of target species (*U. crassus*, *P. phoxinus* and *C. gobio*) to the project area in the Lower Suså is not recommended to be carried out before habitat restoration has been carried out and until the system stabilized for at least one year. It is assumed that the planned river restoration transforms about 1-2 km river (between Holløse Mølle and Skelby) into suitable habitat for re-introduction of the target species (Fig. 23).

However, river locations for species re-introduction in the Lower Suså have to be re-evaluated post restoration. Nevertheless, based on river inventories (the ecological status of Suså, *U. crassus* abundance and fish abundance, see above), there are at least two river locations in the **Lower Suså,** below the dam, that seem to represent suitable habitats for re-introduction of the target species at present, namely Omløb OS Holløse and Holløse Bro (Fig. 22, Table. 5). If no river restoration can take place in the Lower Suså, these two river locations may be targeted for species re-introduction. Here, habitat suitability still depends on the impact of *D. polymorpha* to re-introduced mussels.

Table 5. Summary of potential locations for species re-introduction in the Lower Suså. The names of the river locations correspond to locations shown in Fig. 22 and in Appendix II. Information on the ecological status of Suså (DFI, Danish Physical Index ; DVFI, Danish Stream Fauna Index) is extracted from Birkolm Hansen and Wiberg-Larsen (2017). Information about fish species in the river is extracted from Gørtz and Mouillet (2017). Other information derives from Schneider and Zülsdorff (2017a) and personal communication with experts.

Location	Benefits	Disadvantages and risks
Omløb OS Holløse Bro (N9)	- DFI: good/moderate - DVFI: 5 (8 pos., 1 neg..); 34 species/groups; - Variable water current and water depts - Potential host fish species for *U. crassus*: *P. fluviatilis*, *L. lota*	- Regulated watercourse - Potential clogging of sediments due to planned habitat restoration. - Low substrate heterogeneity - *D. polymorpha* abundance - Green algae cover
Holløse Bro (UC10; N10)	- DFI: good - DVFI: 6 (12 pos, 3 neg.); 56 species/groups - Variable water current and water depts. - Trees and the bridge provide shadow - Food for fish: *Gammarus pulex* - Former home range of *C. gobio* - Potential host fish species for *U. crassus*: unknown	- Potential clogging of sediments due to planned habitat restoration. - *D. polymorpha* abundance - Artificial substrate

Table 6. Summary of river location in the Upper Suså considered less suitable for species re-introduction prior to habitat restoration, however considered more suitable post restoration. A ranking is made for the locations depending on their evaluated suitability for species introduction. RANK 1 represents the location evaluated most suitable. The names of the river locations correspond to locations shown in Fig. 21 and in Appendix II. Information on the ecological status of Suså (DFI, Danish Physical Index ; DVFI, Danish Stream Fauna Index) is extracted from Birkolm Hansen and Wiberg-Larsen (2017). Information of fish species is extracted from Gørtz and Mouillet (2017). Other information derives from Schneider and Zülsdorff (2017a) and personal communication with experts.

RANK	LOCATION	BENEFITS	DISADVANTGAGES AND RISKS
1	**Råen, N f Hjelmsølille** (Ø9)	- DFI: high - DVFI: 5 (10 pos., 3 neg..); 43 species/groups; clear water indicator species present - Natural river course - Stable, heterogeneous substrates of gravel and stones - Variation in flow regime - Macrophyte abundance; shelter for fish - Food for fish, e.g. *Gammarus pulex* - Potential host fish species for *U. crassus*: *P. fluviatilis*, *R. rutilus, L. lota*	- Parts of green algae
2	**Nymølle Bro** (Ø7)	- DFI: high - DVFI: 5 (11 pos., 4 neg..); 43 species/groups; clear water indicator species present - Stable substratum of gravel and stones. - Food for fish, e.g. *Gammarus pulex*	- High abundance of green algae - Potential host fish species for *U. crassus*: unknown

TABLE 6. CONTINUED

3	**Syd for Egebjerg** (Ø10)	- DFI: good - DVFI: 5 (10 pos., 4 neg..); 42 species/groups; clear water indicator species present - Natural river course - Stable bottom substratum of stones and gravel - Food for fish, e.g. *Gammarus pulex*	- High abundance of green algae - Potential host fish species for *U. crassus*: unknown
4	**UC8**	- Presence of at least one living *U. crassus* found upstream of the bridge - High presence of macrophytes (*Potamogeton spp.*) representing shelter for fish - Shadow from the bridge - Variation of bottom substratum incl. large stones	- High abundance of algae - High loads of fine sediments below macrophytes - Deep river areas in which monitoring measures are difficult to carry out - DFI and DVFI: unknown - Potential host fish species for *U. crassus*: unknown
5	**Teestrup Bro** (UC2; Ø3)	- DFI: good/moderate - DVFI: 5 (9 pos., 3 neg..); 44 species/groups; clear water indicator species present - Trees and the bridge provide shadow - Intermediate heterogeneity of sediment - Food for fish, e.g. *Gammarus pulex*	- High abundance of green algae (*Cladophora spp.*) - Presence of nutrient indicator macrophytes (e.g. *Elodea spp.*) - Unlikely a spawning ground for fish - Potential host fish species for *U. crassus*: unknown
6	**Lunden Skov** (UC6)	- Shadow from trees of the forest on the left river side looking in upstream direction - Small riffle - Substratum dominated by pebbles and cobbles	- Very shallow: risk for drought - Difficult to monitor - DFI and DVFI: unknown - Low sediment heterogeneity - Potential host fish species for *U. crassus*: unknown
7	**Granskiftegaard** (UC3; Ø4)	- DFI: good/moderate - DVFI: 4 (11 pos., 3 neg..); 52 species/groups; clear water indicator species present - Trees and the bridge provide shadow - Food for fish, e.g. *Gammarus pulex* - Potential host fish species for *U. crassus*: *P. fluviatilis*, *R. rutilus*	- Clogged and artificial sediments - Presence of green algae and of nutrient indicator macrophytes - No findings of living mussel and shells - Low variation in flow
8	**N f Klintebjerggård** (UC7, Ø8)	- DFI: moderate - DVFI: 4 (9 pos., 4 neg..); 35 species/groups - High variation in flow regime - Food for fish, e.g. *Gammarus pulex* - Potential host fish species for *U. crassus*: *P. fluviatilis*, *R. rutilus, L. lota*	- Poor ecological status poor due to soft sediment dominated by sand and mud - High abundance of green algae and nutrient tolerant macroinvertebrate species - Negative trend of habitat quality - Unlikely to be a good spawning ground for fish

6.1 River locations for the release of infested P. phoxinus

The selection of river locations for the release of *P. phoxinus* infested with glochidia of *U. crassus* should be based on the habitat needs of the fish and the mussel species. For instance, *P. phoxinus* requires well oxygenated gravel banks for spawning and shelter for hiding from predators (see section 1.2). Likewise, juveniles of *U. crassus* are particularly dependent on high habitat quality in the river bottom (see section 1.1). Therefore, fish release in the ***Lower Suså*** is recommended to only be carried out below the dam of Holløse Mølle, at Holløse Bro and at the Omløb OS Holløse Bro (Fig. 22), if no river restoration can take place in the Lower Suså. However, if river restoration can be carried out, river locations located upstream of Holløse Mølle may be targeted additionally (see section 6). Importantly, the release of infested *P. phoxinus* should start only one year post river restoration as fish fry and juvenile mussels falling off the host fish are sensitive to unstable sediments.

Table 7. River location in the Lower Suså considered less suitable for species re-introduction prior to habitat restoration, however considered more suitable post restoration. The names of the river locations correspond to locations shown in Fig. 23 and in Appendix II. Information on the ecological status of Suså (DFI, Danish Physical Index ; DVFI, Danish Stream Fauna Index) is extracted from Birkolm Hansen and Wiberg-Larsen (2017). Information about fish species in the river is extracted from Gørtz and Mouillet (2017). Other information derives from Schneider and Zülsdorff (2017a) and personal communication with experts.

Location	Benefits	Disadvantages and risks
Locations between Stridmøllehus and OS Holløse Mølle (N1-8; incl. UC9)	- Supposed to be good habitat for all target species post river restoration: higher flow velocity; substratum composition adapted to species habitat preferences	- DFI: 13-31 (not transformable to quality as locations are too deep) - DVFI: 4-5 (5-7 pos., 1-4 neg..); 24-40 species/groups - Lake adapted benthic fauna reflects impoundment of the river - High abundance of *D. polymorpha* - Deep water renders monitoring measures difficult to carry out - High numbers of fine sediments and homogenous sediment - Even after river restoration the system needs time to adapt to river conditions

In the ***Upper Suså***, where *P. phoxinus* has its former (and present) distribution, the release of infested fish is recommended to be carried out at the river locations introduced in section 6 and described more in detail in Table 4. However, further river locations, such as presented in Table 6 (e.g. RANK 1-4) can be targeted additionally, although these locations may not represent the overall most suitable river stretches. This is because *P. phoxinus* is a pelagic fish species with high swimming activity, enabling the fish to move within the river searching for the most suitable habitat, if not met upon release. The release of *P. phoxinus* at multiple river locations may increase the chance for successful species re-introduction of both, fish and mussels as more habitat alternatives are provided. Moreover, a release of overwhelming numbers of fish individuals at one river location is avoided in this way. The release of *P. phoxinus* to the Upper Suså can start in the years 2018/2019, after habitat improvement, if any, was carried out (see section 7).

6.1 Locations for re-introduction of C. gobio

The former distribution of *C. gobio* in the River Suså is described to be located downstream of Holløse Mølle, near Herlufholm and Maglemølle (Bollerup, 2015). No former distribution of this fish species is know for the Upper Suså. Therefore, re-introduction of the species to the ***Lower Suså*** has an historical background and is recommended to be carried out downstream of Holløse Mølle, at Holløse Bro and at the Omløb OS Holløse Bro (Fig. 22; Table 5).

However, from an environmental point of view, introduction of *C. gobio* in the ***Upper Suså*** may result in higher re-introduction success. This is because river restoration is planned to be carried out in the Lower Suså during the years 2019-2020, which reduces the feasibility of re-introduction of *C. gobio* to this river part during that time and until the habitat stabilized. Hence, the period for re-introduction of *C. gobio* in the Lower Suså is limited to one project year, if the re-introduction measure is not prolonged for at least two more years. In contrast, re-introduction of *C. gobio* in the **Upper Suså** can start in the year 2018, after minor, if any restoration measures have been carried out (see section 7). Generally, it is recommended to place out *C. gobio* at locations with high habitat heterogeneity, including variations in flow, temperature regime and bottom substratum. We suggest this, as compared with the pelagic *P. phoxinus*, the benthic *C. gobio* has a relatively low migration potential. Hence, re-introduced individuals should have the possibility for choosing among microhabitats. The river locations NS Assendrup, Eskildstrup Møllebro and N f Aversi (Fig. 20; Table 4) are considered most suitable for re-introduction of *C. gobio* in the Upper Suså.

6.2 River locations for translocated mussels and reared juveniles

In the need of implementation of alternative/supplementary conservation strategies to reach the project goals, locations for re-introduction of translocated mussels or reared juvenile mussels are required. Both, adult and juvenile mussels represent relatively stationary life stages that are dependent on high microhabitat quality (see section 1). Therefore, it is recommended to use the most suitable river locations for re-introduction of the mussels. Due to the present threat of *D. polymorpha* in the ***Lower Suså***, this river part may be excluded for re-introduction of reared juveniles or translocated *U. crassus*. However, if a pilot study conducted with living *U. tumidus* or mussel shells shows low colonization of the invasive mussel on the native mussels, the river locations considered most suitable may be targeted (Holløse Bro and Omløb OS Holløse Bro; Fig. 22, Table. 5).

In the ***Upper Suså***, the river locations near the bridge at Assendrup, at Eskildstrup Møllebro, and north of Aversi are considered most suitable for re-introduction of juvenile mussels and of adult mussels directly translocated from a source population to the River Suså (Fig. 20, Table. 4). In particular, the river stretch near Assendrup represents an important location for the translocation of adult mussels, as genetic mixing between introduced and local mussels, located upstream of the location can take place (Appendix II.1).

7. Recommendation for habitat restoration in the River Suså

Habitat restoration represents a major part of the project *UC LIFE Denmark* and encompasses four project actions (C1-C4) planned to take place in the Lower Suså:

- C1 Improvement of bottom substratum in the river
- C2 Planting of riparian vegetation providing shade along the river
- C3 Removal of migration barriers in the river to re-establish river connectivity
- C4 Planting of macrophytes in the river

These actions aim to improve river habitat quality considerably and rendering feasible the re-introduction of *U. crassus* and its affiliated host fish in the Lower Suså. Actions C1, C2 and C4 greatly depend on action C3, which is why C3 is critical for the project. If action C3 cannot take place, adaptive management is required and alternatives to improve habitat quality are needed. Regarding ecosystem benefits, it may even be essential to target habitat restoration measures to additional or other project areas, such as the Upper Suså, where relatively small habitat improvements can result in a large biological benefit and higher feasibility for species re-introduction. We here present such habitat restoration measures to improve habitat quality in the Upper Suså, in particular (section 7.1). However, these improvements can also be carried out in the Lower Suså, particularly if action C3 cannot be implemented. A detailed technical description of the habitat restoration in the River Suså is subject of a specific management plan (Action 1), which should be based on the habitat requirements of *U. crassus*, *P. phoxinus* and *C. gobio*, elaborated in section 1 of this text.

7.1 Specific measures to improve habitat quality

Relatively small river habitat improvements can result in a considerable gain in ecosystem functioning. Such can be the addition of woody debris to the river, an increase of bottom substratum heterogeneity and of variation in the flow regime. In the Upper Suså, we recommend to conduct location-specific improvements prior to the re-introduction of *U. crassus*, *C. gobio* and *P. phoxinus*, as this may increase the success of the conservation strategies.

7.1.1 ADDITION OF WOODY DEBRIS

Large and small woody debris, which is trees or tree branches, have been shown essential shelters for fish hiding from predators, e.g. piscivore fish and birds (Orpwood et al., 2008). In particular, small fish species, such as *P. phoxinus* and also fish fry benefit from woody debris as shelter reduces stress levels and metabolic rates in fish (Millidine et al., 2006; Langford et al., 2012). Moreover, woody debris represents habitat for macroinvertebrate species, which in turn represent food for fish (Hilderbrand et al., 1997). The measure is relatively low in costs and has proven to be more efficient than the implementation of elaborated changes of the river structure (Gustafsson, 2017). We therefore recommend to place out woody debris to river locations where *P. phoxinus* and *C. gobio* is released to Suså.

7.1.2 VARIATION IN SUBSTRATUM HETEROGENEITY AND FLOW REGIME

Heterogeneity of bottom substratum positively affects microhabitat diversity and the variation of the flow regime in a river. As described in section 1 of this text, such is important for *P. phoxinus* and *C. gobio*. It is therefore recommended to place out substratum of different grain sizes, including large boulders, to create backwaters, riffles and hiding places for fish. Backwaters are also essential for mussels, because fine organic matter deposits at low flowing river parts and represents food for the bivalves. Habitat improvement in terms of increasing substrate heterogeneity and flow variation may be carried out in the Upper Suså, north of Assendrup, at Eskildstrup Møllebro and Lunden Skov, as well as at Granskiftegaard and north of Klintebjerggård.

7.1.3 IMPROVEMENT OF SUBSTRATE QUALITY

Gravel (16-32 mm) may be added to river locations with poor spawning ground conditions for *P. phoxinus*, such as at Granskiftegaard and north of Klintebjerggård in the Upper Suså. At Granskiftegaard, loosening up of the sediment is moreover recommended as penetration resistance seemed high (Schneider and Zülsdorff, 2017a). Importantly, this measure should not negatively affect river locations downstream of the measure. Clogging of sediments may also occur in the Lower Suså at Omløb OS Holløse Bro and Holløse Bro after river restoration is carried out upstream of these locations. It is recommended to investigate substrate quality one year after the river restoration.

7.1.4 RIVER STRUCTURE

Retention zones, floodplains and buffer zones to fields are essential structures in a river and affect nutrient retention and availability, as well as erosion. Although the project does not encompass the construction of such river structures in the Upper Suså, it is recommended to preserve what is left (e.g. buffer zones at the location north of Aversi and a natural river shape at Råen and at Nymølle Bro). Maintenance of the river structure should also encompasses prevention of dredging in the river, which however has been carried out in the past and has largely been stopped at present. A measure to improve the riparian zone of the river is the planting of trees, such as planned for the Lower Suså. Trees stabilize the river benches and provide shadow, hence stabilize water temperature in the river. Planting of trees is recommended for river stretches with low tree abundance, such as downstream of Eskildstrup Møllebro and at the location north of Klintebjerggård.

7.1.5 WATER QUALITY

At present, green algae and macrophytes indicating high nutrient loads occur downstream of tributaries to Suså, such as downstream of Assendrup (see Tables 4 and 5). To improve water quality in the River, it is recommended to reduce nutrient loads from effluents of tributaries as much as possible. This however, may be a long process encompassing improvement of waste water management and reduction of local runoffs from fields, and may therefore reach beyond the feasibility of the project *UC LIFE Denmark*.

8. Overall recommendations

During the course of this management plan, a variety of recommendations is provided for the practical implementation and the monitoring of the conservation strategies presented. We here provide a bullet list to summarize the most important aspects mentioned. It is recommended to

Preparation phase

- remove remaining threats that have been causing the decline of the target species at first.
- evaluate the water quality measurements taken during the course of the project.
- conduct additional mussel inventories (wading and diving) for *U. crassus* in the River Suså and Torpe Kanal.
- sample DNA from mussels in Torpe Kanal and from Fyn (Odense Å and Hågerup Å) to confirm the species *U. crassus* genetically.
- conduct population genetic analyses facilitating the selection of suitable source populations of the target species (*U. crassus*, *P. phoxinus* and *C. gobio*).
- compare environmental parameters between donor and recipient rivers for selection of suitable source populations for species re-introduction in the River Suså, particularly if population genetic analyses cannot be conducted.
- communicate with authorities to agree on source populations and species re-introductions.
- investigate survival and/or colonization of translocated mussels (*U. tumidus*) or mussel shells by *D. polymorpha*.
- start organizing equipment requires for the practical implementation of conservation strategies early.

Optional preparation

- test for host fish suitability of farmed *P. phoxinus* prior to introduction to the River Suså (artificial infestation of fish at a laboratory and quantification of juvenile metamorphosis success).
- test for host fish suitability of other fish species present in the River Susså (electrofishing, artificial infestation and quantification of juvenile metamorphosis success).

Practical implementation

- implement conservation strategies according to plan, however being prepared for environmental changes affecting to course of actions (adaptive management; exit strategy)
- administer an excellent communication between project parts to match the timing of successive/parallel conservation measures (e.g. river restoration, fish farming, mussel brood collection, artificial infestation, public outreach).
- re-introduce species after habitat improvement has been carried out, if planned at a certain river location and in downstream direction of such.
- tag mussels collected for brood collection from source populations prior to return to the wild.
- release *P. phoxinus* infested with mussel larvae at multiple times and locations during a project year.
- re-introduce *C. gobio* to Susså without artificial infestation with mussel larvae.
- tag adult mussels if translocated to the River Susså.

Monitoring

- evaluate the success of artificial infestation of host fish (e.g. gill examination).
- evaluate the success of juvenile metamorphosis on host fish (e.g. gill examination of fish maintained in fish cages placed out in the river).
- investigate the survival of fish released to the river (e.g. fish cages, quantitative electrofishing).
- evaluate the survival of translocated *U. crassus*/*U. tumidus* if translocation of mussels was implemented.
- conduct a mussel inventory at project sites about five years post introduction (after LIFE) to evaluate the success of the project.

9. Time schedule for conservation strategies

Successful species conservation requires careful timing of conservation measures to the target species' phenology and ecology, both depending on environmental conditions, such as water temperature. Therefore, the timing of mussel reproduction, hence of brood collection and artificial infestation of host fish can vary. However, the reproduction season of *U. crassus* usually starts in April and gravid mussels can be found in nature between the end of April and the end of June/the beginning of July (section 1, Fig. 24). To better predict the beginning and end of the mussel reproduction season, it is recommended to place out data loggers in streams, and hereby constantly measure water temperatures during spring and summer. High water temperatures in spring and summer can imply an early and short mussel reproduction season. Nevertheless, it is assumed that artificial infestation of host fish can be carried out between the end of April and June.

Fishing at a source population of *P. phoxinus* representing the brood stock (n ~ 300) for farming and release of this fish species to Suså, and for translocation of about 300 adult individuals to Suså (year 2018) is recommended to be carried out during April and May, before local mussel populations released their larvae (Fig. 24). Moreover, it is recommended to split the take up of brood stock for farming of the fish between the years 2018 and 2019, where a number of 150 fish are targeted in each year. In this way, different age classes of fish with increased genetic variance can be farmed and later released to Suså (Table. 8). Alternatively, a number of 300 fish can be caught at one fishing event in 2018.

If the above species re-introduction strategies are not-applicable or fail for some reason, alternative strategies proposed in section 4 can take place in a laboratory (juvenile collection: May-August) and in the field (translocation of adult mussels: May-October). The translocation of *C. gobio* is recommended to be conducted in the fall, when fish condition is highest after reproduction and natural glochidia infestation, if fish derive from mussel rivers (see section 3.3.1). As recommended earlier in the text, monitoring strategies (e.g. tests for host fish suitability, juvenile metamorphosis success, survival of infested fish in the river, survival of translocated mussels) should be carried out during the summer and/or fall, with timing depending on the monitoring approach (Fig. 24).

Importantly, heavy river restoration measures should take place prior to any species re-introduction to ensure that

1) restoration measures do not destroy habitats where newly re-introduced species settled,
2) river substratum has been stabilizing post restoration implying that the survival of juvenile and adult life stages of mussels and fish is not negatively affected by high levels of erosion, and that
3) monitoring of mussels and fish is not disturbed.

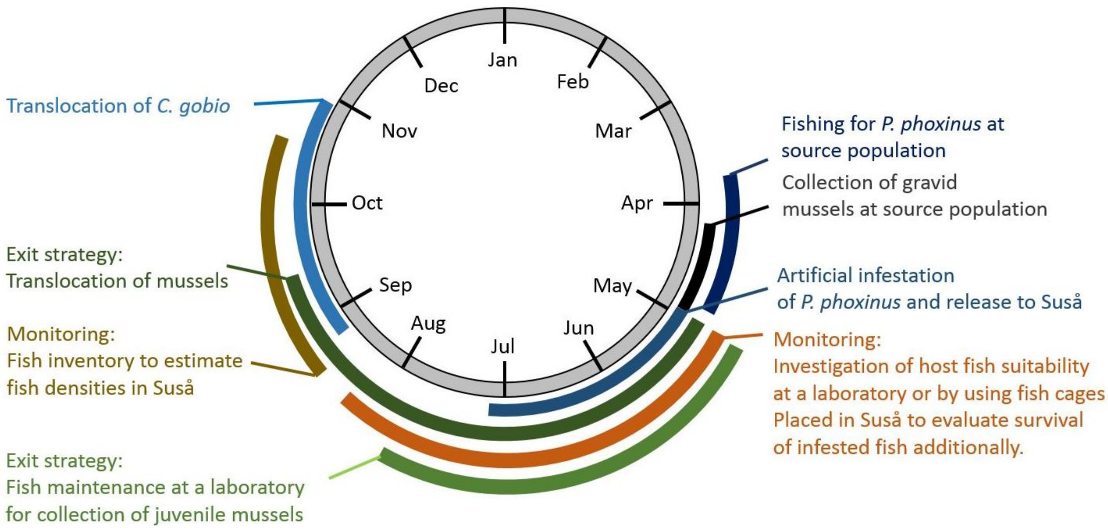

Fig. 24 Suggested time schedule (time wheel) showing recurrent conservation measures (actions, exit strategy and monitoring) of *UC Denmark*.

Næstved Municipality is planning to conduct habitat restoration actions in the **Lower Susâ** during summer and autumn 2019-2020. Species re-introduction in the Lower Susâ should therefore only be carried out later in the project. In the **Upper Susâ** species re-introduction can start in the year 2018, after habitat improvements, if any took place in the river (e.g. improvement of substratum heterogeneity and flow regime, and addition of woody debris, section 7).

A more detailed proposal of the time course of conservation measures is presented in Table 8. Here, recurrent conservation measures shown in figure 24 are listed for every year and project action. Moreover, further actions recommended in this management plan (e.g. DNA-sampling of mussels in Danish rivers and population genetic analyses) are listed. The schedule can be used as a checklist during the practical implementation of the project. Finally, two additional time schedules including detailed suggestions for the implementation of alternative conservation strategies (e.g. mussel translocation and captive breeding of juvenile mussels, Table 9) and monitoring (Table 10) are provided.

Table 8. Proposed time schedule for actions conducted during the years 2018-2021. Legend for coloration:

U. crassus	*P. phoxinus*	*C. gobio*	Genetic analyses	*U. tumidus*	*P. phoxinus + C. gobio*

YEAR	MONTH	ACTION	COMMENTS	CHECK
2018	04-08	Additional search for *U. crassus* in the River Susâ and in Torpe Kanal; aggregation of mussels within each river (not mixing between rivers until population genetic analyses have been conducted).	Highly recommended.	
2018	03-05	Collection of DNA samples from adult *U. crassus* and from fish in Susâ, Torpe Kanal, Odense Å and Hågerup Å.	For population genetic analyses of mussels and fish (comparison of fish/mussels between Fyn, Sjælland and Skåne).	
2018	03-10	Population genetic analyses of mussels and fish and decision on source populations.	Highly recommended.	
2018	03-04	1st fishing of *P. phoxinus* at source population (n ~300, for translocation to the Upper Susâ; and n ~150, for farming at a fish hatchery)	Fish should be caught before the glochidia release of freshwater mussels in the wild, if fishing is conducted in rivers in which unionid mussels exist. The translocation of fish to the Upper Susâ should be carried out at locations upstream of potential habitat restoration measures.	

TABLE 8. CONTINUED

2018	08-10	Fishing for *C. gobio* (n=375) at source population and translocation to the River Suså.	Fish should be caught long after the glochidia release of freshwater mussels, if present at the source population of fish.	
2019	03-04	2nd fishing for 150 *P. phoxinus* at source population for farming the fish at a fish hatchery (2nd year).	Fish should be caught before the glochidia release of freshwater mussels, if present at the source population of fish.	
2019	04-07	Collection of gravid *U. crassus* from source population, transfer to lab and collection of glochidia for artificial infestation of fish.	Adult mussels and glochidia 'left overs' are returned to the wild.	
2019	05-07	Infestation of farmed *P. phoxinus* (n ~ 10000) with *U. crassus* glochidia and release to Suså.	If intended, fish cages in river for fish monitoring.	
2019	08-10	Fishing for *C. gobio* (n ~ 375) at source population and translocation to the River Suså.	Fish should be caught long after the glochidia release of freshwater mussels, if present at the source population of fish.	
2020	04-07	Collection of gravid *U. crassus* from source population, transfer to lab and collection of glochidia for artificial infestation.	Adult mussels and glochidia 'left overs' are returned to the wild.	
2020	05-07	Infestation of farmed *P. phoxinus* (n ~ 15000) with *U. crassus* glochidia and release to Suså.	If intended, fish cages in river for fish monitoring.	
2020	08-10	Fishing for *C. gobio* (n ~ 375) at source population and translocation to the River Suså.	Fish should be caught long after the glochidia release of freshwater mussels, if present at the source population of fish.	
2021	04-07	Collection of gravid *U. crassus* from source population, transfer to lab and collection of glochidia for artificial infestation.	Adult mussels and glochidia 'left overs' are returned to the wild.	
2021	05-07	Infestation of farmed *P. phoxinus* (n ~ 15000) with *U. crassus* glochidia and release to Suså.	If intended, fish cages in river for fish monitoring.	
2021	08-10	Fishing for *C. gobio* (n ~ 375) at source population and translocation to the River Suså.	Fish should be caught long after the glochidia release of freshwater mussels, if present at the source population of fish.	

Table 9. Proposed time schedule for alternative conservation measures conducted during the years 2018-2021 to be able to fulfil the project aims. Legend for coloration:

U. crassus	*P. phoxinus*

YEAR	MONTH	ACTION	COMMENTS	CHECK
2019	04-08	Translocation of adult *U. crassus* (gravid;non-gravid) from source population to Suså (1st year).		
2019	04-07	Collection of gravid *U. crassus* from source population, transfer to lab and collection of glochidia for artificial infestation.	Adult mussels and glochidia 'left overs' are returned to the wild.	
2019	05-07	Infestation of farmed *P. phoxinus* with *U. crassus* glochidia and transfer to the laboratory.		
2019	05-07	Collection of juvenile mussels from infested fish in an aquaria lab facility and rearing in the lab until 2021 or releasing to the river in the same year.	Monitoring of released juveniles in the same year possible via adjusted Withlock-Vibert Boxes.	
2020	04-08	Translocation of adult *U. crassus* (gravid;non-gravid) from source population to Suså (2nd year).		
2020	04-07	Collection of gravid *U. crassus* from source population, transfer to lab and collection of glochidia for artificial infestation.	Adult mussels and glochidia 'left overs' are returned to the wild.	
2020	05-07	Infestation of farmed *P. phoxinus* with *U. crassus* glochidia and transfer to the laboratory.		
2020	05-07	Collection of juvenile mussels in an aquaria lab facility and rearing in the lab until 2021 or releasing to the river in the same year.	Monitoring of released juveniles in the same year possible via adjusted Withlock-Vibert Boxes.	

TABLE 9. CONTINUED

2021	04-07	Collection of gravid *U. crassus* from source population, transfer to lab and collection of glochidia for artificial infestation.	Adult mussels and glochidia 'left overs' are returned to the wild.	
2021	05-07	Infestation of farmed *P. phoxinus* with *U. crassus* glochidia and transfer to the laboratory		
2021	05-07	Collection of juvenile mussels in an aquaria lab facility and releasing to the river in the same year	Monitoring of released juveniles in the same year possible via adjusted Withlock-Vibert Boxes.	
2021	09	Translocation of farmed mussels at the laboratory to the river Suså	(PIT) Tagging of mussels if size allows.	

Table 10. Proposed time schedule for monitoring strategies to be able to follow the success of conservation actions conducted during the years 2018-2021/2024. Legend for coloration:

U. crassus	*P. phoxinus*	*C. gobio*	*U. tumidus*	*P. phoxinus + C. gobio*

YEAR	MONTH	ACTION	COMMENTS	CHECK
2018	05-09	Translocation of *U. tumidus* from the Upper to the Lower Suså to investigate survival and colonization by *D. polymorpa.*		
2019	04-08	Investigation of survival of translocated *U. tumidus* and the level of colonization by *D. polymorpha*.		
2019	05-07	Monitoring of exit strategy: Investigation of fish survival in cages placed out to the river to follow and evaluation of the level of glochidia encapsulation.		
2020	04-08	Monitoring of exit strategy: Investigation of survival of translocated *U. crassus* (from 2019, 1st year) and the level of colonization by *D. polymorpha*.		
2020	05	Investigation of host fish presence (*P. phoxinus + C. gobio*) in Suså.	Quantitative electrofishing.	
		Monitoring of exit strategy: Investigation of fish survival in cages placed out to the river to follow and evaluation of the level of glochidia encapsulation.		
2021	04-08	Monitoring of exit strategy: Investigation of survival of translocated *U. crassus* (from 2019+2020) and the level of colonization by *D. polymorpha.*		
2021	05	Investigation of host fish presence (*P. phoxinus + C. gobio*) in Suså.	Quantitative electrofishing.	
		Monitoring of exit strategy: Investigation of fish survival in cages placed out to the river to follow and evaluation of the level of glochidia encapsulation.		
2024	04-08	Monitoring of exit strategy: Investigation of survival of translocated *U. crassus* (from 2019+2020) and the level of colonization by *D. polymorpha.*	After LIFE.	
2024	04-08	Investigation of *U. crassus* abundance in the River Suså.	After LIFE.	
2024	05	Investigation of host fish presence (*P. phoxinus + C. gobio*) in Suså.	After LIFE.	

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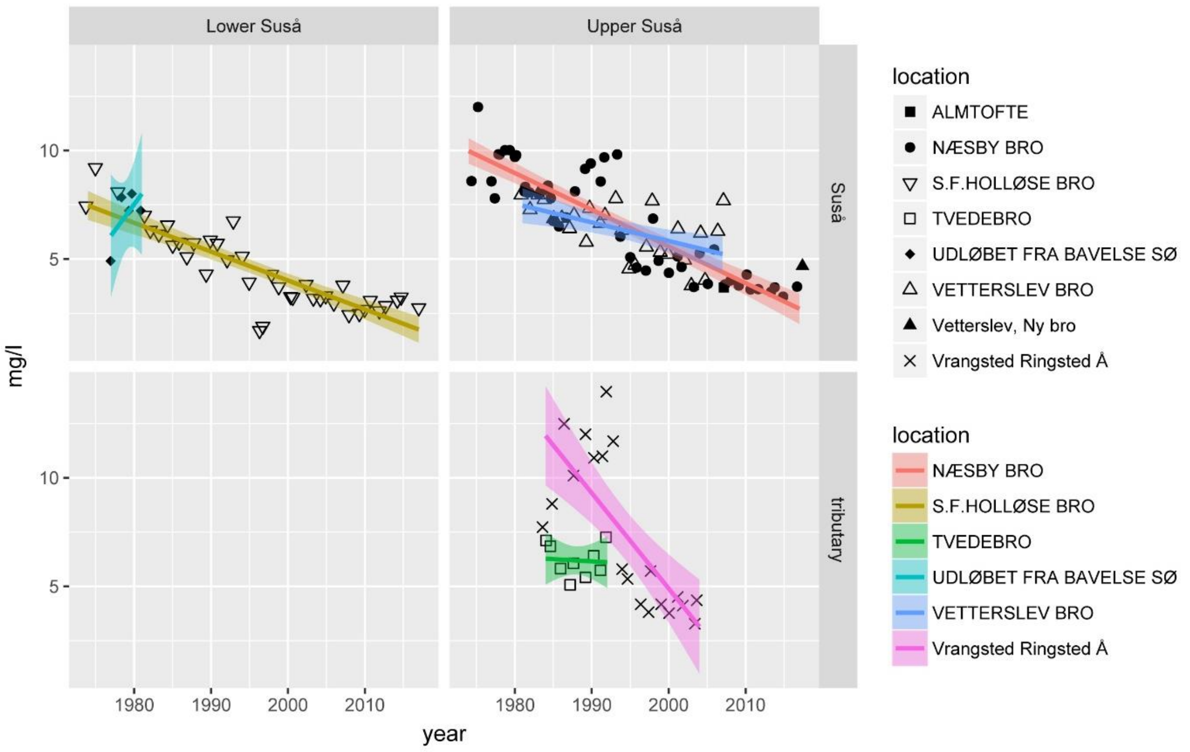
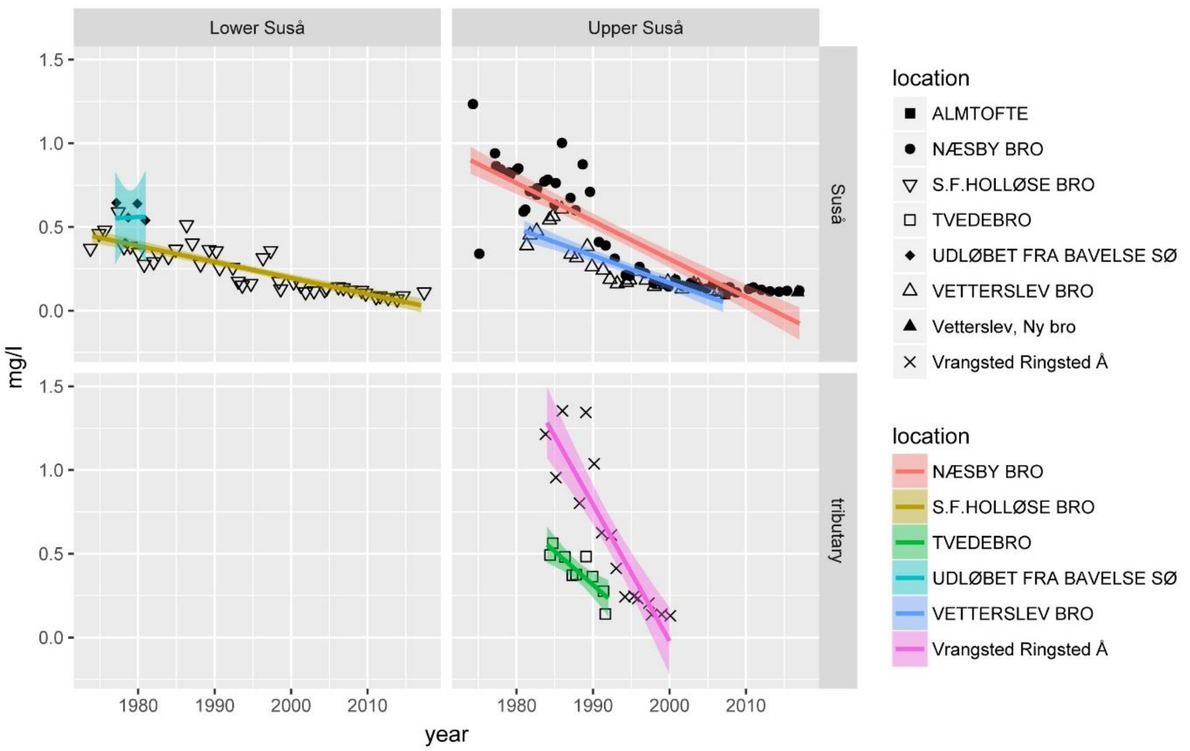
Appendix I: Water chemical parameters in the River Suså and tributaries

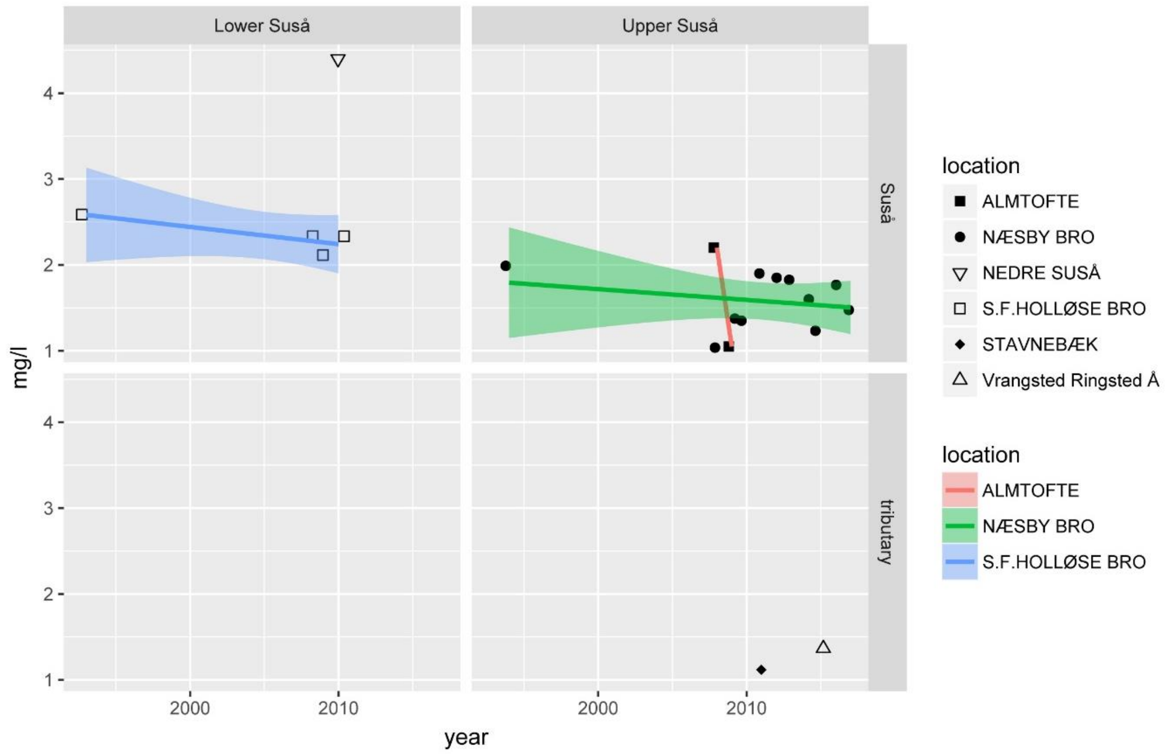
Total nitrogen



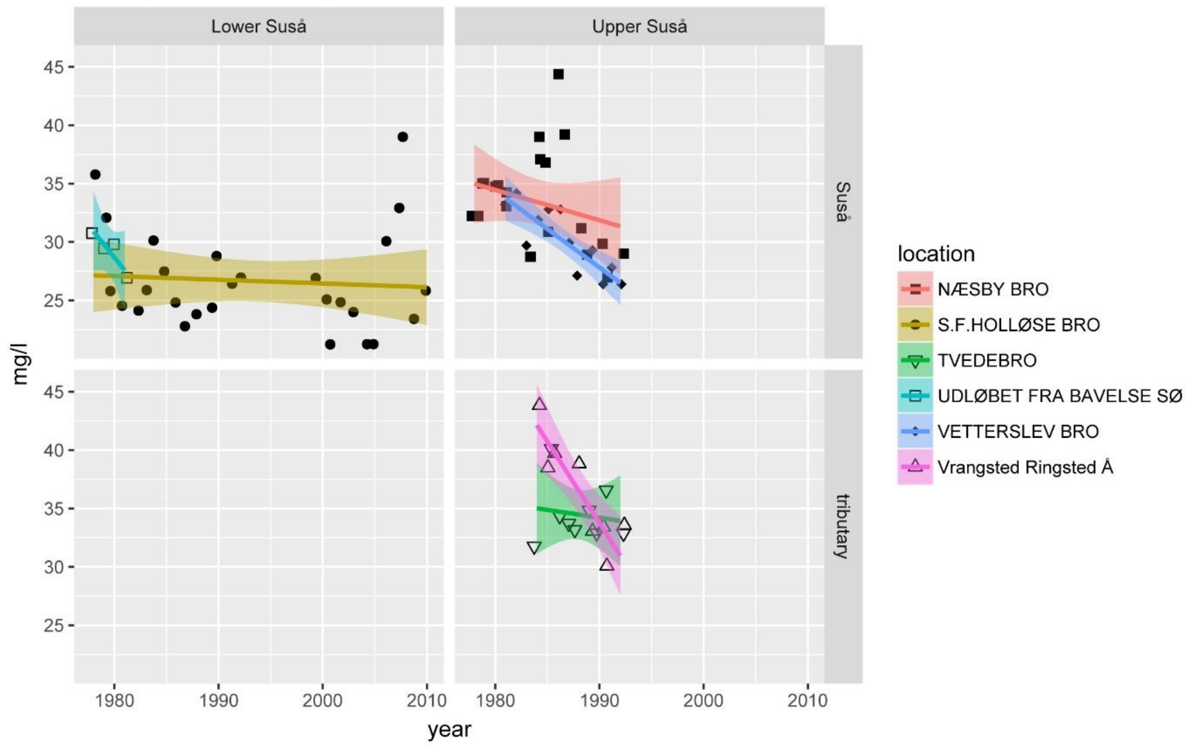
Total phosphorous



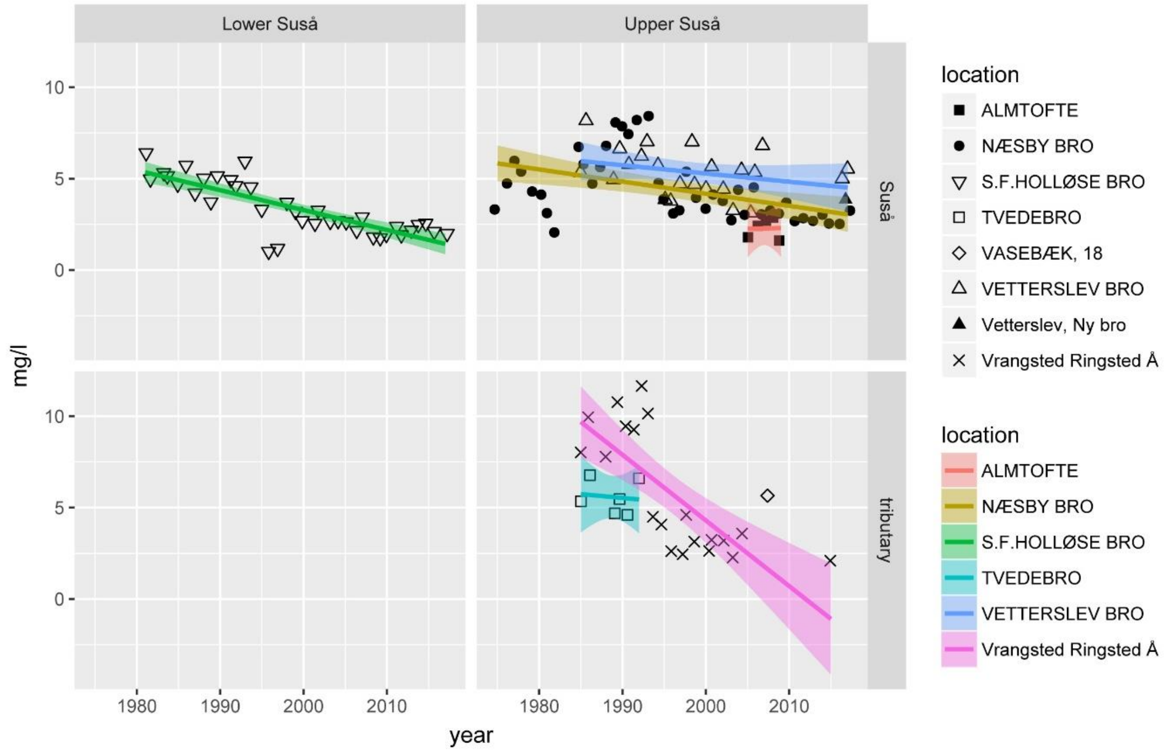
Biological oxygen demand in 5 days (BOD_5)



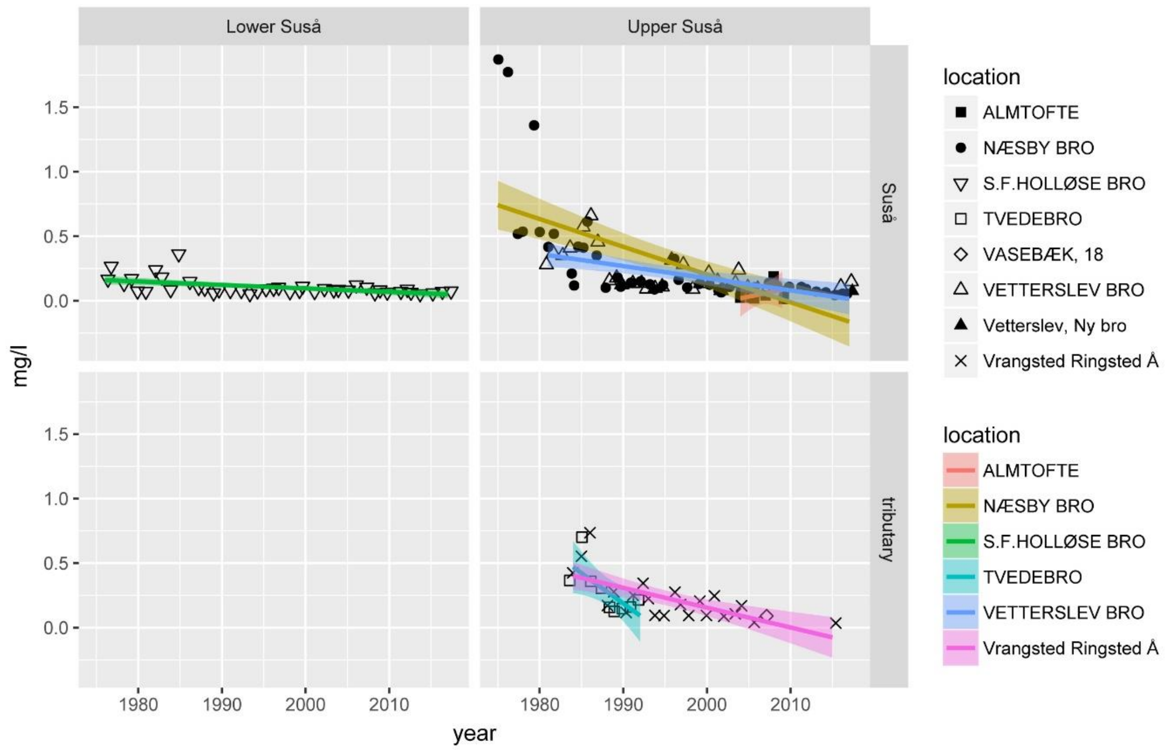
Chemical Oxygen Demand (COD)



Nitrite nitrate nitrogen (NO_2-NO_3-N)

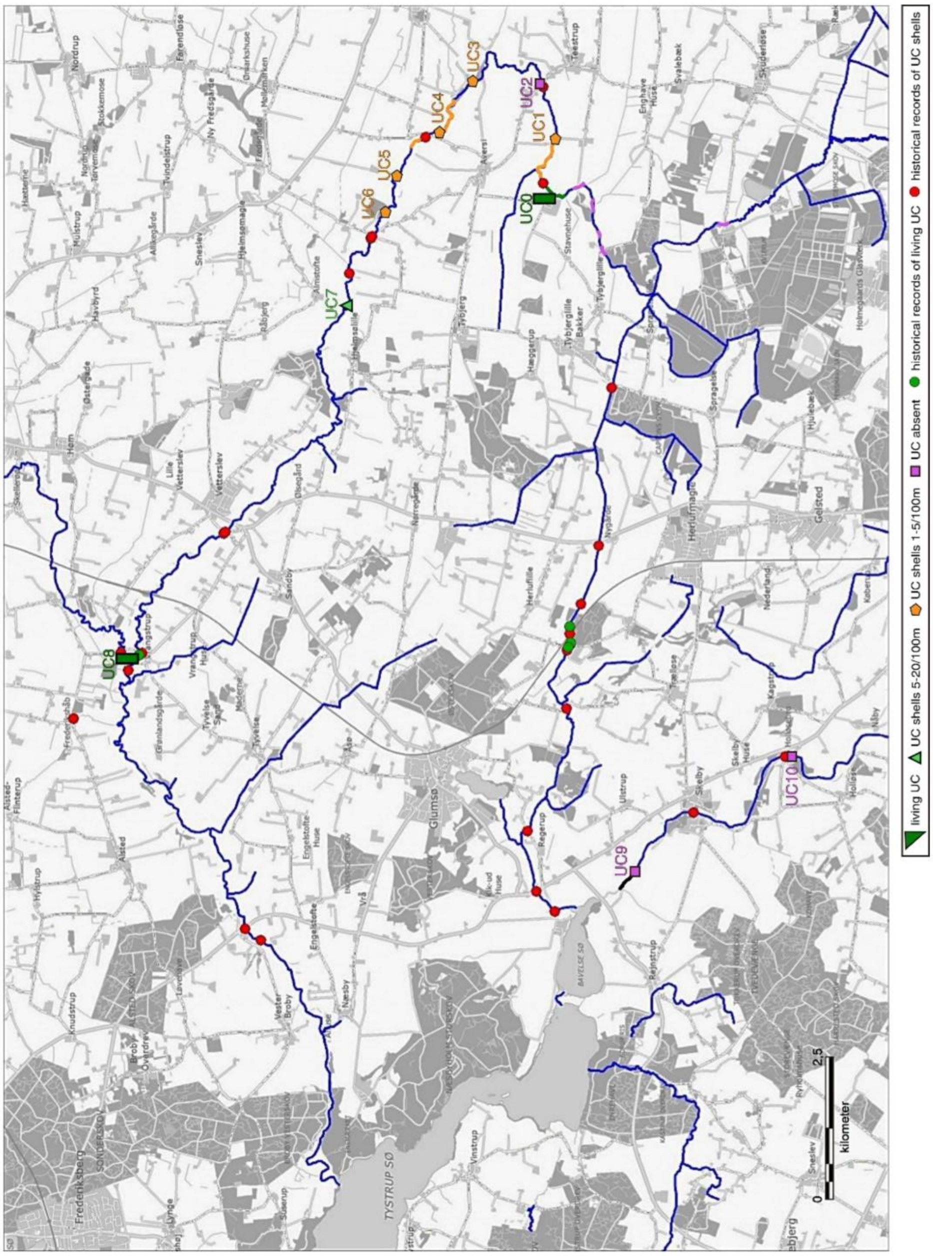


Ammoniak ammonium nitrogen NH_3-NH_4-N

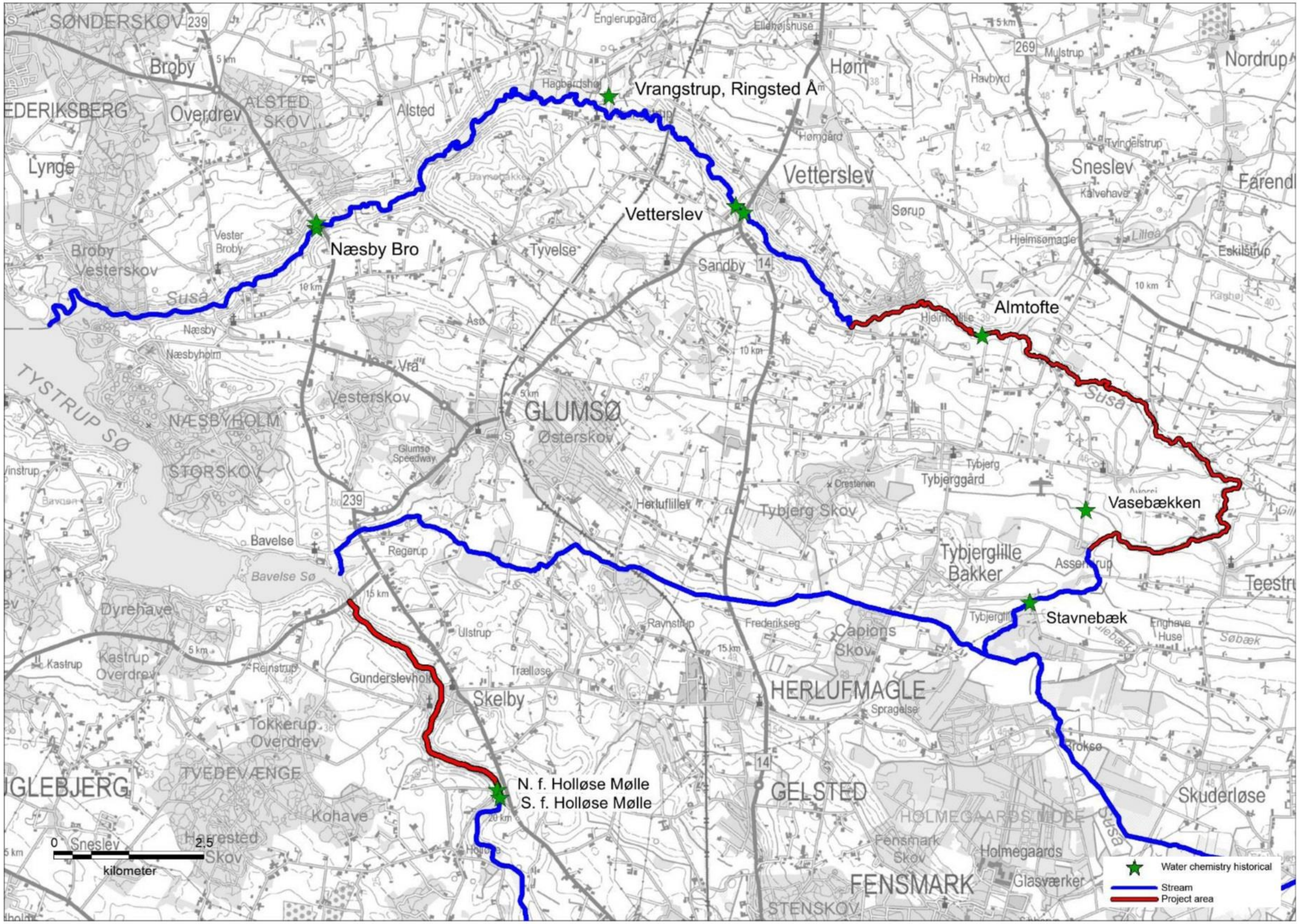


Appendix II: Additional maps

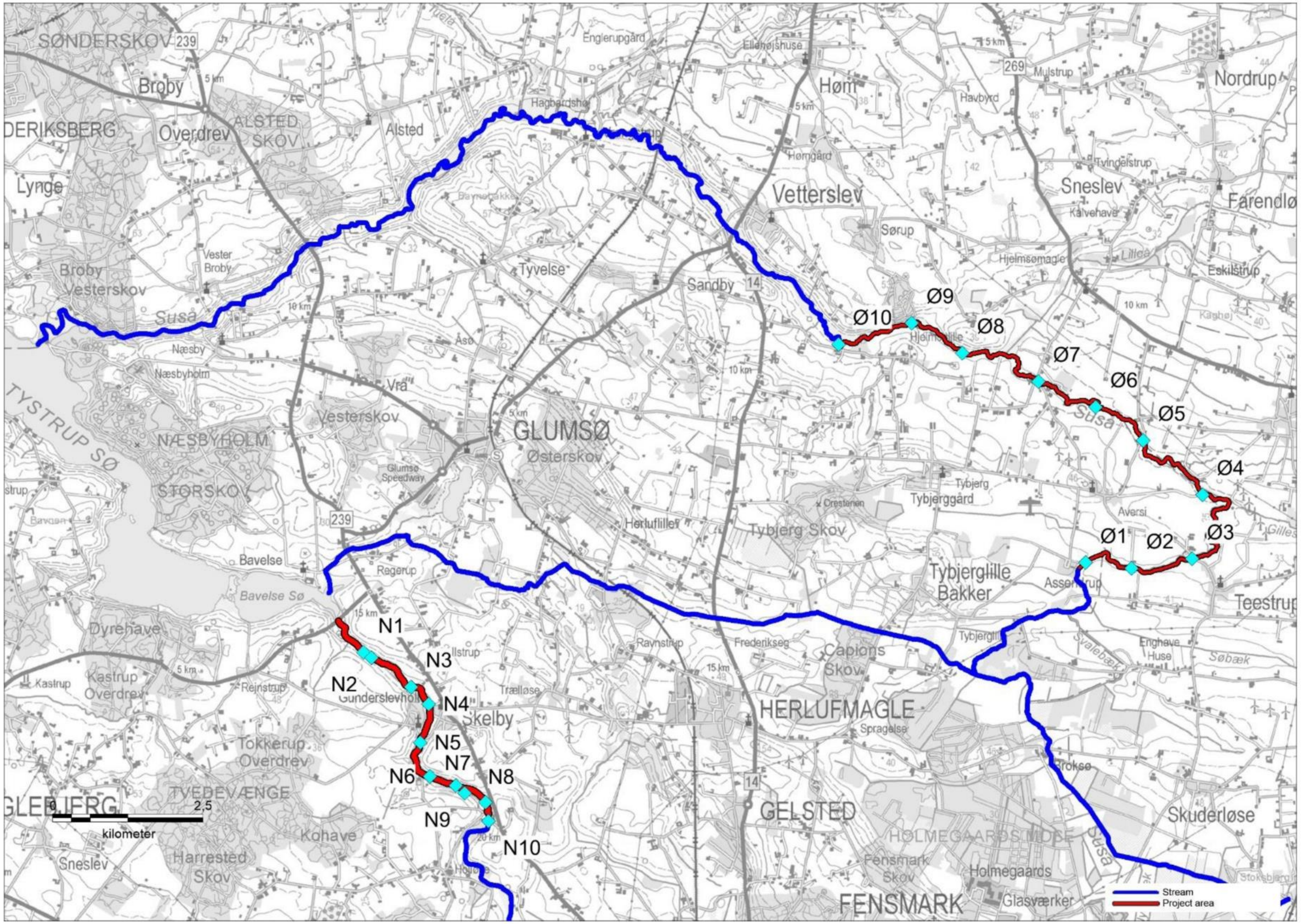
Appendix II.1 Overview map of the River Suså and of Torpe Kanal showing historical findings of living *Unio crassus* and shells (green and red circles) and findings from the present mussel inventory. Symbols denote the *U. crassus* inventory sites in Suså. Lines show river stretches of additional screening for *U. crassus*. Dark green bars and lines denote findings of living *U. crassus* individuals and purple squares the absence of living *U. crassus* and shells. Light green triangles show the presence of *U. crassus* shells at densities of 5-20 shells/100m, and orange polygons at densities of 1-5 shells/100m. From Schneider and Zülsdorff (2017a).



Appendix II.2 River locations at which water chemisty measurements were carried out in the past. The map was kindly provided by Næstved Municipality.



Appendix II.3. River locations at which the ecological status (DFI, DVFI) of the river Suså was evaluated in 2017. The map was kindly provided by Næstved Municipality.



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