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Follow-up investigation of *Unio crassus* in Suså post conservation efforts

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Summary

A qualitative mussel investigation including non-area specific excavation of sediment was carried out in the River Suså post re-introduction efforts conducted for the thick-shelled river mussel *Unio crassus* and its affiliated host fish the European minnow (*Phoxinus phoxinus*) during the LIFE project UC LIFE Denmark (LIFE15NAT/DK/000948). The investigation resulted in the detection of bothshells and living specimens of the mussel species *Unio tumidus* and *U. pictorum* of various sizes and ages, with the smallest living individual of *U. tumidus* holding a shell length of 20 mm. However, none of the living specimens were represented by *U. crassus*. Albeit the absence of *U. crassus* in this investigation, the results are not implying the failure of re-introduction efforts for the species, but rather underline the difficulty to detect mussels in river systems where infested fish were released freely. Moreover, 57 % of the mussels re-introduced via their host fish are today, if survived, no older than 2 years, whereof 27 % were no older than three months. Therefore, future mussel investigations on a regular basis starting in about 2-4 years are recommended in order to better estimate the outcome of the conservation efforts.

1 Background

One of the main goals of the European LIFE project UC LIFE Denmark (LIFE15NAT/DK/000948) was to improve the status of the threatened thick shelled river mussel *Unio crassus* in the River Suså, on Zealand – Denmark. Previous mussel inventories showed that only a few specimens are left in the river at two locations namely *Assendrup* and *Vrangstrup* (figure 1). In order to reach this goal and to re-introduce the European minnow, a primary host fish for *U. crassus*, to Suså simultaneously, minnows with origin in the River Hågerup å on Fyn, were artificially infested with mussel larvae from the same river and released at 11 locations in the upper Suså during 2019-2023 (figure 1, table 1). Habitat restoration such as replacement of larger stones which have been removed in earlier times and addition of gravel for re-establishment of spawning grounds went along with the described measures.

In 2019, the first 2000 minnows were infested with *U. crassus* and released at the two locations *Lunden* and *Teestrup Bro* (table 1). In 2020, 3100 minnows were released at the same locations and at *Assendrup*; these three locations were also targeted in 2021 where 10 000 minnows were released in total. In 2022 and 2023, 10 000 minnows each, where released at seven other locations in Suså, namely *Almstofte*, *Assendrup Hgd*, *Broksø Bro*, *Næsby Bro*, *Næsby Kano*, *Sørup Hgd* and *Vrangstrup* (figure 1). Taking in consideration the year of fish release, the oldest mussels possibly surviving post fish release should therefore occur at *Lunden* and *Teestrup Bro* with an age of 3-4 years. Taking in consideration the number of fish released at each location, the same locations (*Lunden* and *Teestrup Bro)* as well as *Sørup Hgd,* should habitat the highest number of possibly surviving mussels. However, fish are mobile and move among habitats in river systems which renders difficult statements on exact locations on which juvenile mussels may have fallen off the fish after their 3-4 weeks long metamorphosis. It is further not guaranteed that habitat conditions at locations where juvenile mussels drop of the fish meet the mussels' requirements. Nevertheless, it is important to monitor and evaluate the outcome of the conservation measures conducted, at locations considered optimal for the target species.

In the present follow-up, a qualitative mussel monitoring including non-area specific excavation of sediment was conducted at four river locations in the River Suså. This investigation was meant to get an insight in the present status of *U. crassus* in Suså at this relatively early stage post conservation efforts.

Figure 1. Overview of locations in the River Suså at which I) the mussel inventory took place in 2017 (UC0-UC8, Schneider & Zülsdorff (2017)*, and II) infested minnows (Phoxinus phoxinus) were released during the project between 2019-2023.*

Table 1. *Overview of locations in the River Suså at which infested minnows were released during the project between 2019-2023. Coordinates: EPSG: 25832 – ETRS89/UTM zone 32N. Data provided by Næstved municipality.* ID according to Schneider & Zülsdorff (2017).

Location		Coordinates		Infested minnows released during the project					
Name	**ID**	**x**	**y**	**2019**	**2020**	**2021**	**2022**	**2023**	**Total**
Almstofte		678392,19	6139368,47					2500	2500
Assendrup	1 - UC0	680590,68	6135612,63		1100	2500			3600
Assendrup Hgd		680717,09	6135706,86					2500	2500
Broksø Bro		680059,94	6132592,87					2500	2500
Lunden		680411,85	6138360,53	1000	1000	2500			4500
Næsby Bro		667957,48	6141098,47					2500	2500
Næsby Kano		667988,77	6141111,15				2500		2500
Sørup Hgd		677365,66	6139554,08				5000		5000
Sørup/Hjelmsølille		677809,77	6139669,72			2500			2500
Teestrup Bro		682601,48	6135796,24	1000	1000	2500			4500
Vrangstrup	UC8	672538,05	6142803,93				2500		2500
			TOTAL	**2000**	**3100**	**10 000**	**10 000**	**10 000**	**35 1000**

2 Inventory locations

Four river locations were targeted for the present follow-up of re-introduction efforts conducted for the thick-shelled river mussel *Unio crassus* and its host fish, the European minnow *Phoxinus phoxinus* in the River Suså *between 2019-2023* (figure 2, table 1). The locations were selected by Næstved municipality based on habitat quality parameters indicating beneficial conditions for minnows and juvenile mussels, and the year and number of infested fish released at or close by the locations during the project. Hence, the locations were partly overlapping with the locations at which infested minnows were released (figure 2) and at which previous mussel inventories have been carried out (figure 1, Schneider, 2019; Schneider & Zülsdorff, 2017). The inventory locations were between 30-170 m long (table 2).

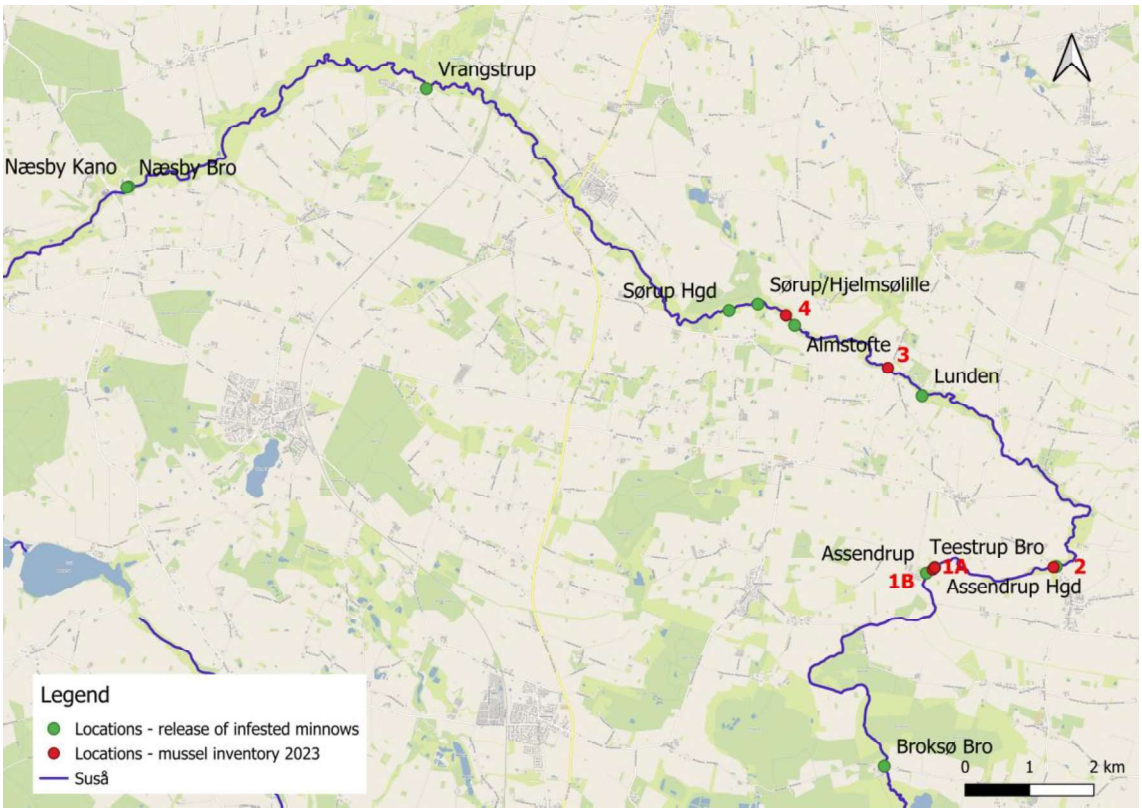

Figure 2. Overview of inventory locations in the River Suså at which I) infested minnows (Phoxinus phoxinus) were released during the project between 2019-2023, and II) the follow-up mussel inventory was conducted in 2023; (1B and 1A) Near Assendrup – UC0, (2) Teestrup bro – UC2, (3) Near Lunden and (4) Near Almtofte.

Location 1 (UC0, figure 3), near Assendrup, was divided into two sections, a 30 m stretch upstream of the bridge (location 1B) and a 35 m long stretch downstream of the bridge (location 1A, Assendrup Hgd. Living individuals of both *U. crassus* and other unionoid mussel species, particularly *U. tumidus* are present in high densities on both stretches but particularly on 1B. Nevertheless, individuals of *U. crassus* are old and sparse. A total of 2500 minnows infested with *U. crassus* larvae was released at 1A and 3600 minnows at about 100 upstream of 1B during three project years (table 1).

Location 2 (UC2, figure 4) reaches from down-to upstream of the bridge close to Teestrup and was about 70 m long. During previous mussel inventories, shell findings indicated the presence of mussels in the past, however no living mussels were found (Schneider & Zülsdorff, 2017). A total of 4500 infested minnows were released at the location during four project years (table 1).

Location 3 (figure 5) is 170 long and reaches from down- to upstream of the bridge close to Lunden. The location lies about 600 and 650 m downstream of the mussel inventory location UC5 (near Lunden) and the fish release location *Lunden* where 4500 infested minnows were released during 2019-2021, respectively.

Location 4 (figure 6) is located about 400 m downstream of the mussel monitoring location UC7 and 140 m downstream of the location *Almtofte*, where 2500 infested minnows were released in 2023. This location was included in the present follow-up as it was found to be a highly attractive habitat for minnows, most likely due to habitat restoration conduced in terms of replacement of larger stones which in turn improved habitat and flow heterogeneity.

Table 2. Overview of inventory locations and methods used.

Location ID	Location ID according to Schneider & Zülsdorff (2017)	Location name	Coordinates WGS84, down	Length (m)	Methods
1A	UC0	Assendrup Hgd – downstream of the bridge	55.334161, 11.848752	30	Visual inspection, 6 x sediment samples
1B	UC0	Assendrup - upstream of the bridge	55.334423, 11.849248	35	Visual inspection, 5 x sediment samples
2	UC2	Teestrup bro	55.33449, 11.878317	70	Visual inspection, 8 x sediment samples
3	-	Near Lunden	55.362253, 11.837811	170	Visual inspection, 6 x sediment samples
4	-	Near Almtofte	55.369534, 11.812849	84	Visual inspection, 5 x sediment samples

Figure 3. Monitoring location 1 (UC0), down- (left) and upstream (right) of the bridge at Assendrup. Pictures are taken in downstream direction.

Figure 4. Monitoring location 2 (UC2), down (left)- and upstream (rigth) of the bridge close to Teestrup. Pictures are taken in upstream direction.

Figure 5. Monitoring location 3, down (left)- and upstream (right) of the bridge close to Nymøllevej. The left picture is taken in downstream direction, the right picture in upstream direction.

Figure 6. Monitoring location 4, down-(left) and upstream (right) of the bridge at Almtofte. Pictures are taken in upstream direction.

3 Methods

The mussel investigation was conducted using two methods (table 2). First, the river bottom was visually inspected by wading in upstream direction using a bottom viewer, collection tongs and buckets/nets for mussels (figure 7). Second, sediment was sampled using 2 mm nets positioned at the river bottom such as when using a surber sampler for benthic fauna, though the sediment upstream of the net was excavated to about 10-15 cm substratum depth and shuffled into the net (figure 7). The sediment was then transferred to white trays for inspection, alternatively sieved into four size fractions (>31,5 mm, 31,5-16,0 mm, 16,0-8 mm, and < 4,0 mm) using laboratory test sieves prior to inspection (figure 8 and 9). Focus was on both living mussels and shells.

Figure 7. Visual inspection of the river bottom (left) and example of net for sampling sediments (right). Pictures by Sofia Mulla Kølmel.

Figure 8. Sediment collection and sieving.

Figure 9. Inspection of sampled river sediment for mussels.

4 Results

Living mussels (Unionoida) were found at locations 1A, 1B, 3 and 4 and were represented by *Unio tumidus* and *U. pictorum* (table 3, figures 10, 11 and 12). The mussels were both visible at the sediment surface and detected in the sediment samples. At the first location (1A and 1B) 6 specimens of *U. tumidus* with a shell length below 25 mm were detected, four in the sediment sample and two during the visible inspection of the river bottom. No living mussel were found at location 2 and no living individuals of *U. c*rassus were found at any inventory location. Other observations were fish, such as minnows, particularly at location 4 and spined loach *Cobitis taenia*, as well as high numbers of small freshwater mussels, such as *Pisidium amnicum*.

Table 3. Overview of mussel findings at each inventory location.

Location	***Unio tumidus***	***Unio pictorum***	***Other observations***
1A	18 living, whereof 1 <25 mm	2 living, 2 valves	*Pisidium spp*
1B	13 living, 4 shells, whereof 5 <25 mm	2 valves	*Pisidium spp*
2	-	-	*Pisidium spp*
3	1 living	-	*Cobitis taenia*, *Pisidium spp*
4	2 living, 2 valves	-	*Phoxinus phoxinus*, *Cobitis taenia*, *Pisidium spp*

5 Discussion and recommendations

The mussel investigation resulted in the detection of living specimens of *Unio tumidus* and *U. pictorum* at three of the four locations examined. Most specimens were found at *Assendrup* and also represented size classes < 25 mm, which is in line with previous findings (Schneider & Zülsdorff, 2017). However, no living *U. crassus* were found in the present investigation. It is however important to mention that the search excluded the specific position at the location *Assendrup* at which *U. crassus* specimens previously detected and tagged were aggregated as their detection was beyond the purpose of this investigation.

However, the results of this investigation, not detecting *Unio crassus*, are not surprising. Difficulties in monitoring and re-capturing of (tagged) mussels, particularly when they are sparsely distributed and buried in the sediment are well known (Bergengren, 2000; Kurth et al., 2007; Lang, 1998; Zieritz et al., 2014). Even the re-catch of tagged mussel by means of visual inspection, PIT (Passive Integrated Transponder) detection and digging can be as low as 25-56 % (Schneider, 2022), but digging generally increases the outcome several fold (Dunn, 2000; Ljungberg, 2019). In this investigation, visual inspection and sampling of sediments down to a depth of 10-15 cm was conducted. Still, the findings are not implying the failure of re-introduction efforts for *U. crassus* in Suså. Besides the difficulties in detecting sparsely distributed mussels, it has to be mentioned that 57 % of the mussels re-introduced to Suså via their host fish are today, if survived, no older than 2 years, whereof half are not older than a few months, hence holding a shell size of about 200-400 µm. Moreover, fish were released to the river freely and juvenile mussels may have fallen of their hosts after their 3-4 weeks long metamorphosis at other locations than targeted in this investigation. It is further not guaranteed that habitat conditions at any location in the river meet the mussels' requirements. However, the findings of small *U. tumidus* at *Assendrup* indicate suitable habitat conditions for juvenile mussels at this location.

Finally, regular investigations in 2-4 years from today are recommended in order to better estimate the outcome of the conservation efforts. The inventories shall take place in the late spring and early summer when vegetation cover is low and adult mussels taking part in reproduction move to the sediment surface (Bauer, 2001; Pfeiffer & Nagel, 2010).

Figure 10. Living mussels and shells of Unio tumidus and Unio pictorum found at location 1A – Assendrup.

Figure 11. Living mussels and shells of Unio tumidus and Unio pictorum found at location 1B – Assendrup.

Figure 12. Living mussels and shells found at location 3 (left) and 4 (right).

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