



Current status of the thick-shelled river mussel *Unio crassus* in the River Suså: Version 2 - including results of DNA analyses

Technical report 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

Background – freshwater mussels

Freshwater mussels (Order: Unionoida) are keystone species in aquatic ecosystems (Vaughn, Hakenkamp 2001). As benthic filter-feeding organisms, they provide important ecosystem functions with significant ability for water filtration, nutrient deposition (bioturbation), sediment mixing and stabilization (Strayer 2008; Limm, Power 2011). However, the long and complex life cycle of unionoid mussels, including a temporary parasitic life stage on fish and a sensitive juvenile live stage which buries in the stream or lake sediment for several years, renders mussels highly vulnerable to anthropogenic habitat changes (Barnhart et al. 2008; Bauer, Wächtler 2001). Major threats to mussels are water pollution, river channelization, damming - also affecting fish, invasive species and overexploitation. Such have been causing drastic declines of mussel populations worldwide since the industrial revolution (Bogan 2008; Lydeard et al. 2004).

*Background – *Unio crassus**

In Europe, the thick-shelled river mussel *Unio crassus* is one of the most endangered freshwater mussel species (Cuttelod et al. 2011). Based on population declines and extinctions all over its distribution area, this species is Red listed and included in the EC Habitats Directive (92/43/ECC) in Appendix II and IV (Bachmann 2000). *Unio crassus* is a stream-dwelling species occurring in upper to middle reaches, together with its host fish. Primary host fish species for the mussel are represented by the European minnow *Phoxinus phoxinus*, the European bullhead *Cottus gobio*, the European Chub *Squalius cephalus*, the three-spined stickleback *Gasterosteus aculeatus* and the common bleak *Alburnus alburnus* (Lopes-Lima et al. 2017). These fish species can, however, be accompanied or supplemented by secondary host fish, such as the burbot *Lota lota*, the ruffe *Gymnocephalus cernua* and the European perch *Perca fluviatilis*. However, host fish suitability and availability varies between watersheds depending on fish community pattern and abundance, and renders the mussel-host fish interaction unique for every river system (Schneider 2017). At any rate, a lack of suitable host fish can lead to population declines and extinctions (Zale, Neves 1982).

Background - UC LIFE Denmark

The European LIFE project *Actions for improved conservation status of the thick-shelled river mussel (*Unio crassus*) in Denmark (LIFE15NAT/DK/000948)* was established in order to improve the habitat quality and status of *Unio crassus* and its host fish in the River Suså, Næstved municipality. Agricultural runoff, channelization and dredging have been negatively affecting the natural channel morphology and ecology of the river, hence the presence of *U. crassus* (Pedersen et al. 2004). Declines of *P. phoxinus* and the extinction of *C. gobio* have enforced the pressure on the mussel species, together with an invasion of the zebra mussel *Dreissena polymorpha* in the Lower Suså (Jensen 2001). Current knowledge is that only few individuals of *U. crassus* are remaining in the river (Miljøstyrelsen 2017).

Historical mussel findings in the River Suså

Between the years 2007 and 2015, the Danish Environmental Protection Agency (Miljøstyrelsen) performed 13 mussel surveys distributed over 23 localities in the River Suså and in Torpe Kanal (Appendix I). One living individual of *U. crassus* was found in the River Suså, near Vrangstrup on

10 June 2015. In the years 2008, 2009 and 2015, a number of three, seven and five living *U. crassus* were found at multiple locations in Torpe Kanal. Ringsted – Femern Banen (2014) conducted a mussel survey in Torpe Kanal and reported 14 living *U. crassus*. Hereof, a number of six living *U. crassus* were found in a small investigation between Ravnstrupvej and the railway bridge of the Ringsted-Vordingborg connection on 13 June 2017 conducted by Schneider, Zülsdorff (2017). During all investigations, shells of *U. crassus* were recorded in both, the Upper and Lower Suså and in Torpe Kanal (Appendix I).

Purpose and implementation of the mussel inventory

Knowledge about the current status of the thick-shelled river mussel *U. crassus* in the River Suså is essential for river restoration measures, including the improvement of habitat quality for *U. crassus* and its host fish, and the re-establishment of the species. Moreover, information about the population size is needed to estimate the natural reproduction potential of *U. crassus*, which also is affected by the availability of local host fish. Hence, knowledge about the status of local host fish (e.g. Gørtz, Mouillet 2017) is essential for the establishment of conservation strategies such as mussel propagation and re-introduction.

The mussel inventory run in August 2017 encompassed overview screening for *U. crassus* at 11 river stretches of 50 to about 100 m length, in both the Upper and Lower Suså. Additionally, one survey site was investigated in detail (specific 20 m-inventory). Further river sites were point sampled. All living *U. crassus* found were PIT tagged and aggregated in the river.

The following sections of this report are guiding through the measures conducted during the mussel inventory, including study area, methodology, a detailed presentation of all locations investigated, and result discussion.

Study area

The River Suså represents one of Denmark's largest waterways (drainage area: 835 km², total length: 83 km). It is located in the geomorphologically and hydrologically eastern part of Denmark, on the island Zealand (Danish: Sjælland), in agricultural areas on glacial deposits, hence represents a lowland river (Thodsen 2007). It originates in Tingerup Tykke, south of Rønnede and is part of the municipalities Næstved, Faxe and Ringsted (Mothiversen, Thorup 1987). The River Suså is divided in two parts, the Upper Suså, supplied by several small tributaries, and the Lower Suså mouthed in the bay of Karrebæksminde (Fig. 1). Near Næstved, the average discharge is 7 m³ s⁻¹.

The inventory sites were chosen according to historical records of *U. crassus* in Suså and by means of on-site evaluation of most suitable habitats and the presence of mussel shells and other living mussel species. In the Upper Suså, six survey sites were distributed between Assendrup and Almtøfte (referred as to UC0-UC7), and one at Vrangstrup (UC8, Fig. 4). River stretches upstream of Assendrup and between UC0 and UC7 were point sampled additionally. In the Lower Suså, one survey site was located at Stridmøllehus (UC9) and one at Holløse Mølle (UC10, Fig. 4).



Figure 1. Map of the upper (Øvre) and lower (Nedre) part of the River Suså.

Methods

The mussel inventory was conducted in line with the national instructions for the monitoring of *U. crassus* in Denmark (Wiberg-Larsen 2015).

Overview Screening

Overview screenings took place at the inventory sites UC0-UC10 and followed the instructions of Wiberg-Larsen (2015), point 2.3.2 'Udlægning af prøvefelter til screening af tilstedeværelse' and point 2.3.3 'Den praktiske registrering ved screening', appendix 6.1a. GPS coordinates (WGS 84) of survey sites were taken on the right river shore looking in downstream direction. Survey sites were about 100 m long with variation depending on local condition. Mussel abundance was investigated by wading through the river using aquascopes. Here, the river bottom substrate was visually inspected. Shells and living individuals were collected, species identified and documented. The mussel abundance was extrapolated to a 100 meter river stretch for all present mussel species. The smallest individual of *U. crassus* was measured in length [nearest \pm mm]. Living individuals of *U. crassus* were stored in net bags in the river until tagged. For all survey sites, the length, the average depth and width were measured and sediment heterogeneity and composition of sand (0.06 – 2 mm), granules and pebbles (2 – 60 mm), and cobbles and stones (60 – 600 mm) estimated. Further parameters, such as shading, river regulation, and the presence of macrophytes, algae and fish were noted additionally. The survey sites were reached via direct access from land (UC8, UC10) or by canoe (UC0-UC7) and boat (UC9).

Specific investigation

A specific mussel investigation was conducted at the survey site UC0 following the instructions by Wiberg-Larsen (2015), point 2.3.4 'Udlægning prøvefelter: undersøgelse af tæthed og aldersstruktur', appendix 6.1a,b. On a river stretch of 20 m, the sediment bottom was screened for living *U. crassus* and shells in proceeding one meter river length at a time. Measuring tape placed parallel and orthogonal to the river shore ensuring precision (Fig. 2). For each 1m-investigation, living mussels and shells were carefully picked from the sediment for species identification and documentation, however shells other than *U. crassus* were collected at half of the river stretch only. As the number of living *U. crassus* at the survey site was lower than 50 individuals, the search for mussels was extended in up- and downstream direction for 20 m and for a maximum of 30 minutes. Identical to the overview screening, survey site parameters were recorded.



Figure 2. Set up and screening for *Unio crassus* during the specific mussel inventory at the survey site UC0.

Additional Screening

The search for *U. crassus* was expanded through point sampling at river stretches between the survey sites UC0- UC7, using an aquascope from boat (canoe) or by wading. Likewise, additional screening was conducted upstream of UC0 until the bridge near Tybjerglille. Habitat parameters, such as macrophyte and algae cover, living mussels and shells were recorded as well as changes in the watercolor. The presence of *U. crassus* in Suså was moreover investigated on a 100 m long river stretch at the bridge of Broksøvej, accessible by car. Several hours of visual and manual (by hand) searching for *U. crassus* was carried out at the river stretch, at which most living *U. crassus* were detected during the monitoring. All living individuals of *U. crassus* were collected in net bags kept in the river until further investigations (size measurements and tagging) took place.

Tagging of mussel individuals

All living *U. crassus* were tagged with an individual Passive Integrated Transponder (PIT, 12 mm, Oregon), which was glued on the mussel shell near the umbo using superglue (Locite) and marine epoxy (Pattex). Individual PIT tag numbers were scanned and noted, together with paper-tag numbers glued besides the PIT tag. Moreover, morphological measurements (length, height, width, nearest \pm mm) were taken and mussels photographed. Mussels of morphology mistakable to the morphology of *U. crassus* were treated likewise and a note was made regarding the species identification. Foot tissue samples were taken of all tagged mussels and stored in 95 % ethanol for DNA analyses. All mussels were placed back to the River Suså near the survey site UC0 where they were aggregated (Fig. 3).



Figure 3. Tagged mussels from the River Suså (A), placed out near Assendrup, close to survey site UC0 (B).

DNA analyses

Species identification of mussels tissue sampled during the inventory in the River Suså was carried out by the Swedish Museum of Natural History in Stockholm using molecular methods (ITS rDNA analyses, Källersjö et al. 2005). The results of the DNA analyses were incorporated in the present (second) version of the report.

Results

During the mussel inventory in the River Suså in August 2017, a total of 6 living individuals of *U. crassus* (genetically confirmed) was found at two survey sites in the Upper Suså - five individuals

at UC0 near Assendrup and one individual at UC8 near Vrangstrup, the latter confirming the historical finding of *U. crassus* at UC8 (Fig. 4, Appendix III). Other mussels DNA sampled and species identification by means of molecular methods represented the swollen river mussel *Unio tumidus* (n = 16) and the painter's mussel *Unio pictorum* (n = 1). No shells or living *U. crassus* were recorded in the Lower Suså, where the invasive zebra mussel *Dreissena polymorpha* represents the dominant mussel species. Shell findings of *U. crassus* were recorded at all but one survey site in the Upper Suså, whereas most shells occurred at the survey site UC0, where *U. crassus* co-exists with a dense population of *U. tumidus* (Fig. 4, Appendix III).

In the following sections of this report, detailed results of the overview and specific mussel investigation are presented for each survey site and inventory approach. In the appendixes II and III, a summary of the data is provided in table form.

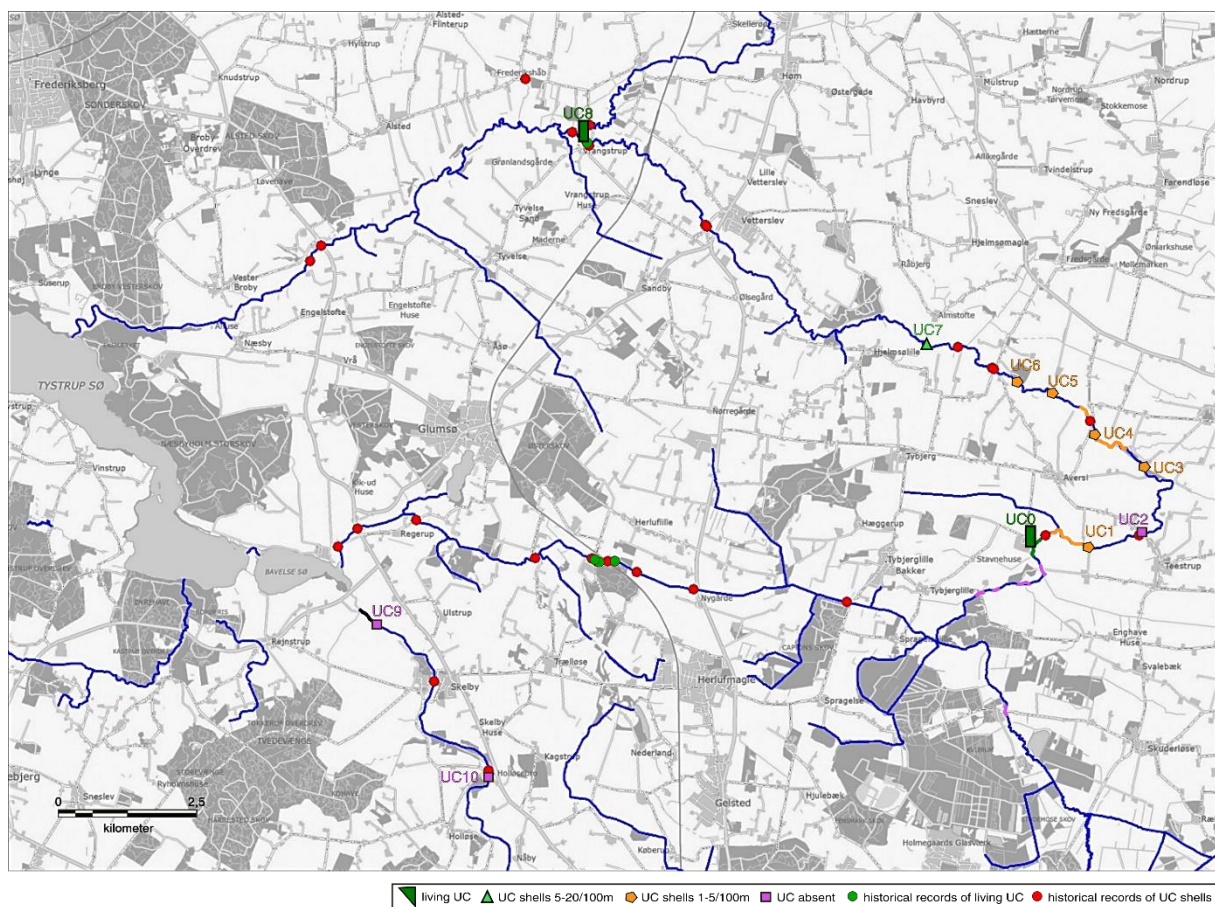


Figure 4. Overview map of the River Suså and of Torpe Kanal showing historical findings of living *Unio crassus* and shells (green and red circles) and findings from the present mussel inventory. Symbols denote the *U. crassus* inventory sites in Suså. Lines show river stretches of additional screening 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.

Upper Suså - survey site UC0 near Assendrup

The survey site (100 m in length) is located 200 m upstream of the bridge near Assendrupvej (WGS 84 decimal (lat, lon): 55.33320, 11.84685), where the river is 5.6 m wide and 0.7 m deep, on average. A forest patch surrounds the river from the bridge to 500 m upstream. The dense tree

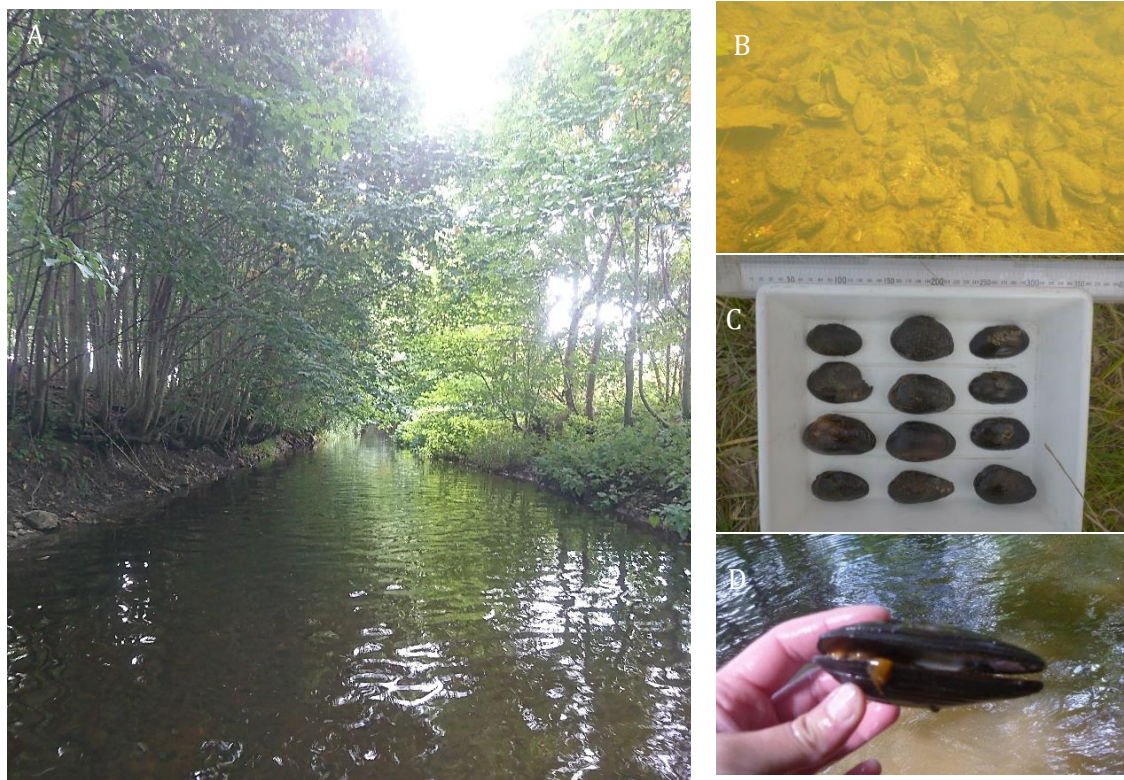


Figure 5. The River Suså near Assendrupvej - UC0 – in upstream direction (A); picture of the river bottom at UC0 (B); shells of *Unio crassus* and *U. tumidus* (C); decaying individual of *U. tumidus* found near UC0 (D).

cover provides shadowing of the river up to 90 % (Fig. 5A). Tree roots stabilize the steep shore, particular at the left riverside looking in upstream direction. The streambed is soft, although dominated by pebbles and cobbles. The sediment heterogeneity is intermediate. A thin layer of organic material covers the sediment (Fig. 5B), but algae are absent. Along the survey site, no artificial river regulation occurs and no dredging took place in the past. Smell of manure from the fields surrounding the forest patch and the river was substantial.

Among a dense population of the swollen river mussel *U. tumidus* (abundance: many > 20 individuals/100m), a number of three living *U. crassus* were found during the overview investigation (abundance: low 1-5 individuals/100 m). Another living *U. crassus* was found at UC0 during the specific investigation at UC0 where a 20 m river stretch was thoroughly screened for mussels. One more individual of *U. crassus* was found during additional screening slightly upstream of UC0. The smallest living individual of *U. crassus* had a shell length of 62 mm (from UC8). The average shell length was 80 mm. The morphology of *U. crassus* varied between individuals and was very similar to *U. tumidus* in some individuals (Fig. 5C and Fig. 19).

Most mussels occurred in the middle to left side of the riverbed looking in upstream direction. A copper rust, clearly visible underwater, covered the majority of the mussels found at UC0, particularly where a large piece of metal laid in the water.

During the overview investigation, shells of *U. crassus* were found at UC0 at intermediate abundance (5-20 shells/100m). Shells of *U. tumidus* were present at high abundance (> 20 shells/100m), of *Anodonta anatina* and of *A. cygnea* at low abundances (1-5 shells/100m, Fig. 5C). During the specific inventory, the shell presence of *A. anatina* and *A. cygnea* was categorized as intermediate (5-20 shells/100m) and high (>20 shells/100m), respectively.

Among many old shells, there were several shells with little or no corrosion. One newly dead individual of *U. tumidus* was found (Fig. 5D).

Upper Suså - survey site UC1 north of Henriettelund

The survey site of 100 m length is located north of Henriettelund (WGS 84 decimal (lat, lon): 55.33314, 11.86280), where the river is surrounded by fields. Single trees (aspen, alder and willow) are present at the shoreline and shadow the river up to 40 % (Fig. 6A). The common reed (*Phragmites australis*) stands on the riversides in the lower part of the survey site. The riverbed is largely covered by macrophytes, particularly the bur-reed (*Sparganium spp.*) below which fine sediment accumulates. No artificial river regulation occurs. On average, the river is 4.6 m wide and 0.4 m deep. The maximum depth is 0.5 m. The heterogeneity of the sediment is intermediate with granules, pebbles and cobbles dominating the bottom substratum.



Figure 6. The River Suså north of Henriettelund – UC1 – in upstream direction (A); mussel shells of *Unio crassus* and *Anodonta cygnea* (B); shells (C) and living individuals of other mussel species (D).

At UC1, shells of *U. crassus* were found at low abundance (1-5 shells/100 m, Fig. 6B), however no living individuals. *Unio tumidus* and *U. pictorum* thrive at intermediate abundances (5-20 individuals/100 m) and high numbers of shells occurred (> 20 shells/100m, Fig. 6C, D). Few shells of *A. cygnea* were found (1-5 shells/100m, Fig. 6B). Most mussels were present under trees, where excavators cannot reach the river for dredging.

Upper Suså - survey site UC2 at the bridge of Teestrup

The 100 m long survey site UC2 reaches from up- to downstream of the bridge of Teestup (WGS 84 decimal (lat, lon): 55.33444, 11.87769, (Fig.7A). On average, the river is 4.5 m wide and 0.4 m deep and has a maximum depth of 0.7 m. In the lower part of the survey site, a small platform occurs next to the river (Fig.7B). No other artificial river regulation is present. However, high abundance of algae cover the riverbed (Fig.7C) with patches occurring from the tributary upstream of UC1 until UC3. The habitat heterogeneity is low and sand dominates the substratum. Trees (alder, willow) provide shadow up to 30 %. Shell findings indicate the presence of mussels in the past (Fig.7D). However, no living mussels were found at present.



Figure 7. The River Suså at the bridge of Teestrup – UC2 – in upstream direction (A); platform located downstream of the bridge (B); algae cover at the river bottom of UC2 (C); mussel shells from UC2 (D).

Upper Suså - survey site UC3 at Granskiftegaard

Down- and upstream of a small river crossing at Granskiftegaard, the River Suså was screened for *U. crassus* on 75 m (WGS 84 decimal (lat, lon): 55.34433, 11.87985). River shore stabilization and debris from the bridge represent artificial river regulation to an intermediate degree (Fig. 8). Patterns of algae occur, the streambed is clogged and substrate heterogeneity is low - granules and pebbles dominate. Macrophytes are present in up- and downstream direction of the survey site, in particular, but small patches of pondweed (*Potamogeton spp.*) occur. Spruce forest shadows the river up to 30 %. The river has an average width and depth of 5.4 m and 0.7 m, respectively. Maximum depth is 0.8 m.

No living mussels were found at the survey site UC3. Mussel shells were represented by *U. crassus* and *A. cygnea*, both occurring at low abundance (1-5 shells/100m), and *U. tumidus* shells are present at intermediate abundance (5-20 shells/100m).

Figure 8. The River Suså at Granskiftegaard – UC3 – in downstream direction.

Upper Suså - survey site UC4 at Eskildstrup Møllebro

The survey site UC4 (100m in length) is located up- and downstream of the bridge at Eskildstrup (WGS 84 decimal (lat, lon): 55.35054, 11.865964). Fields and a four-meter wide riparian buffer zone reach the river on the right side looking in downstream direction. On the left side, bushes and a meadow separate the river from fields (Fig.9A). Single trees of maple, alder and birch grow on the river shore, providing up to 2 % shadow (Fig.9B). In the river, macrophytes (e.g. *Sparganium spp.*, *Potamogeton spp.*, *Elodea spp.*) cover the streambed up to 90 % (Fig. 9C). The sediment heterogeneity is low and fine sediments accumulating in the macrophyte beds cover granules and pebbles, the dominating substrate on UC4. On average, the river is 5.2 m wide and 0.7 deep and has a maximum depth of 0.8 m.

Living mussels were represented by *U. tumidus* (abundance: low 1-5 individuals/100m), exclusively, and the majority of shells belonged to this species (abundance: intermediate 1-5 shells/100 m). Shells of *U. crassus*, *A. cygnea* and *U. pictorum* occurred at abundances of 1-5 individuals/100m (Fig. 9D).



Figure 9. The River Suså at Eskildstrup Møllebro – UC4 – in downstream (A) and upstream direction - picture taken from the bridge (B); macrophyte cover at UC4 (C); mussel shells found at UC4 (D).

Upper Suså - survey site UC5 east of the forest Lunden

The survey site UC5 (100 m in length) is located east of the forest Lunden and north of Aversø (WGS 84 decimal (lat, lon): 55.35804, 11.85326). The survey site starts at a wooden bridge and follows a natural s-shaped winding of the river (Fig. 10A). The river is 4.1 m wide and 0.4 m deep, on average, and has a maximum depth of 0.5 m. At the upper end of the survey site, riparian vegetation ranges wide in the river channel. A willow provides 2 % shadowing. The sediment is dominated by granules, pebbles and cobbles, and has an intermediate heterogeneity. Macrophytes cover the riverbed to a small extent.

A number of five living mussels were found at UC5, all representing small individuals of *U. tumidus*, *A. cygnea* and *A. anatina* with low abundance (1-5 individuals/100m, Fig. 10B, C). No living *U. crassus* was found at the survey site UC5. Shells of *U. crassus* occurred at densities of 1-5 shells/100m and were highly corroded (Fig. 10D).



Figure 10. The River Suså east of the forest Lunden – UC5 – in upstream direction (A); living *Unio tumidus* half-buried in the river bottom at UC5 (B); living mussels (C) and shells (D) found at UC5.

Upper Suså - survey site UC6 at the forest Lunden

South of the forest Lunden, the survey site UC6 (116 m in length) is located (WGS 84 decimal (lat, lon): 55.359618, 11.844678). Here, the River Suså is adjoined by forest at the left side and by fields at the right site, both looking in upstream direction (Fig. 11A). Trees provide shadow up to 35 %. With an average depth of 0.2 m, the river is very shallow. The search for mussels was difficult on the very shallow parts of the survey site as shadow from the aquascopes darkened the riverbed, and ripples on the river surface decreased visibility with the naked eye. In the upper part of the survey site, the river is deeper and has a maximum depth of 0.7 m. Average width of the river is 5.6 m. The sediment is dominated by pebbles and cobbles and has a low heterogeneity. No artificial river regulation occurs. Few macrophytes are represented by *Sparganium spp.* and *Lemna spp.*.

No living *U. crassus* were found at UC6 and shell density was low (1-5 shells/100m). The shells were old and partly overgrown by algae. Living mussels were represented by small individuals of *U. tumidus*, found in the upper part of the survey site (abundance: low 1-5 shells/ 100m). Shells of *U. tumidus* and *A. cygnea* were distributed all over the river stretch and had an abundance of 1-5 shells/100m (Fig. 11B).



Figure 11. The River Suså near the forest Lunden – UC6 – in upstream direction (A); living mussel individuals (*Unio tumidus*) and mussel shells (*U. tumidus*, *U. crassus* and *Anodonta cygnea*) found at UC6 (B).



Figure 12. The River Suså South of Almtofte – UC7 – in upstream direction (A); mussel shells found at UC7 (B).

Upper Suså - survey site UC7 south of Almtofte

South of Almtofte, the survey site UC7 (100 m in length) starts downstream of an S-shaped winding of the river Suså (WGS 84 decimal (lat, lon): 55.367314, 11.817727, Fig. 12A). In the middle-right site of the river stretch a maximum depth of 1.4 m is reached. Average depth and

width are 0.7 m and 5.1 m, respectively. No artificial river regulation occurs, but weeds from river vegetation clear-cuts conducted upstream in the river got caught on stones at the edges. Submerged leaves of *Nuphar lutea* - the yellow water-lily, and *Sparganium* spp. represent the present macrophytes. Shadowing from willow was 5 %. The sediment heterogeneity was low, and granules, pebbles and cobbles form the dominant substratum composition.

Shells of *U. crassus* occurred at intermediate densities (5-20 shells/100m, Fig. 12B). No living mussels were found at UC7.

Upper Suså - survey site UC8 near Vrangstrup

Downstream of the bridge and the landing place for canoes, the start of the survey site UC8 is located (WGS 84 decimal (lat, lon): 55.401121, 11.724665, Fig. 13A). In downstream direction, the river is deep and not suitable for a mussel investigation by wading, as it is upstream of the bridge, limiting the investigated river stretch to a length of 50 meters. Here, the average depth and width of the river is 0.9 m and 7.3 m, respectively. The construction of the bridge and the landing place for canoes represent artificial structures at both the river edges and the river bottom. Together with trees, the bridge provides shadow at 1-5 % of the river stretch, depending on the position of the sun. *Phragmites australis* stands at the edges in the lower part of the survey site. Excessive occurrence of algae cover both the riverbed and the macrophytes (e.g. *Sparganium* spp.). Manual removal of algae was needed to evaluate the sediment composition and to conduct the mussel inventory. The sediment heterogeneity was intermediate with pebbles and granules dominating and large stones distributed in the upper part of the inventory site. Fine sediment occurred all along the river stretch, particularly underneath macrophytes.

One individual of *U. crassus* was found right upstream of the bridge (abundance: low 1-5 individuals/100 m, Fig. 13B), confirming the presence of *U. crassus* reported from a former investigation in the year 2015 (Appendix I). A number of six mussel individuals showed a morphology mistakable to *U. crassus*. According to DNA analyses such individuals represent *U. tumidus*. Shell abundance of *U. crassus* was low (1-5 shells/100 m).



Figure 13. The River Suså at Vrangstrup – UC8 – in upstream direction (A); living individuals of *Unio crassus* and *U. tumidus* found at UC8 (B).

The abundance of living *U. tumidus* and shells was intermediate (5-20 individuals/100 m). Living *A. cygnea* was present at low abundance (1-5 individuals/100 m), but no shells were found of this species.

Lower Suså – survey site UC9 at Stridmøllehus

In the Lower Suså, an overview investigation was conducted at a 100 m long river stretch 350 m downstream of Stridmøllehus (WGS 84 decimal (lat, lon): 55.324896, 11.659563, Fig. 14A). The investigation was conducted by wading and by boat. The average depth and width of the river is 0.9 m and 13.9 m, respectively, and the maximum depth exceeds 1.5 m. At the river shores, willows provide 5 % shadow and *P. australis* grows. Submerged *Nuphar lutea* and *Elodea spp.* are present in the water, however not near beaches. Cows have access to the river. Fine sediment and high algae occurrence cover the riverbed (Fig 14B). The substratum is dominated by sand and sediment heterogeneity is low.

Living individuals of large freshwater mussels were absent. However, the invasive zebra mussel *D. polymorpha* (abundance: many >20 individuals/100m) covered all kinds of hard substrates such as stones and mussel shells of the native species *U. tumidus* (abundance: low 1-5 shells/100 m, Fig. 14C, D). Shells of *D. polymorpha* were present at high abundances (many > 20 shells/100 m).

Lower Suså – survey site UC10 at Holløse Mølle

Down-to upstream of the bridge at Holløse Mølle, a 100 m long survey site - UC10 was investigated for mussels (WGS 84 decimal (lat, lon): 55.299911, 11.689625, Fig. 15A). The natural river regulation is affected by the bridge and the mill raceway, as well as by garbage, construction waste and non-native shells of marine mussels and snails (e.g blue mussels and oysters) which are artificial sediments in the river (Fig. 15B). Natural sediments are represented by granules, pebbles, cobbles and stone, rendering an intermediate habitat heterogeneity. Macrophyte cover is patchy and is represented by *Potamogeton spp.*, *N. lutea* and *Myriophyllum spp.* Willow provides 1% shadow on the river. The average depth and width of the river is 0.4 m and 19.8 m, respectively, and maximum depth is over 1.5 m.

Living individuals of native mussels were absent. The invasive zebra mussel *D. polymorpha* occurred at high densities (>20 individuals/100 m) and at some parts its shells covered the riverbed entirely (Fig. 15C, D).

Shells of native mussels were represented by *A. cygnea* and *U. tumidus*, both occurring at low densities (1-5 shells/100 m).

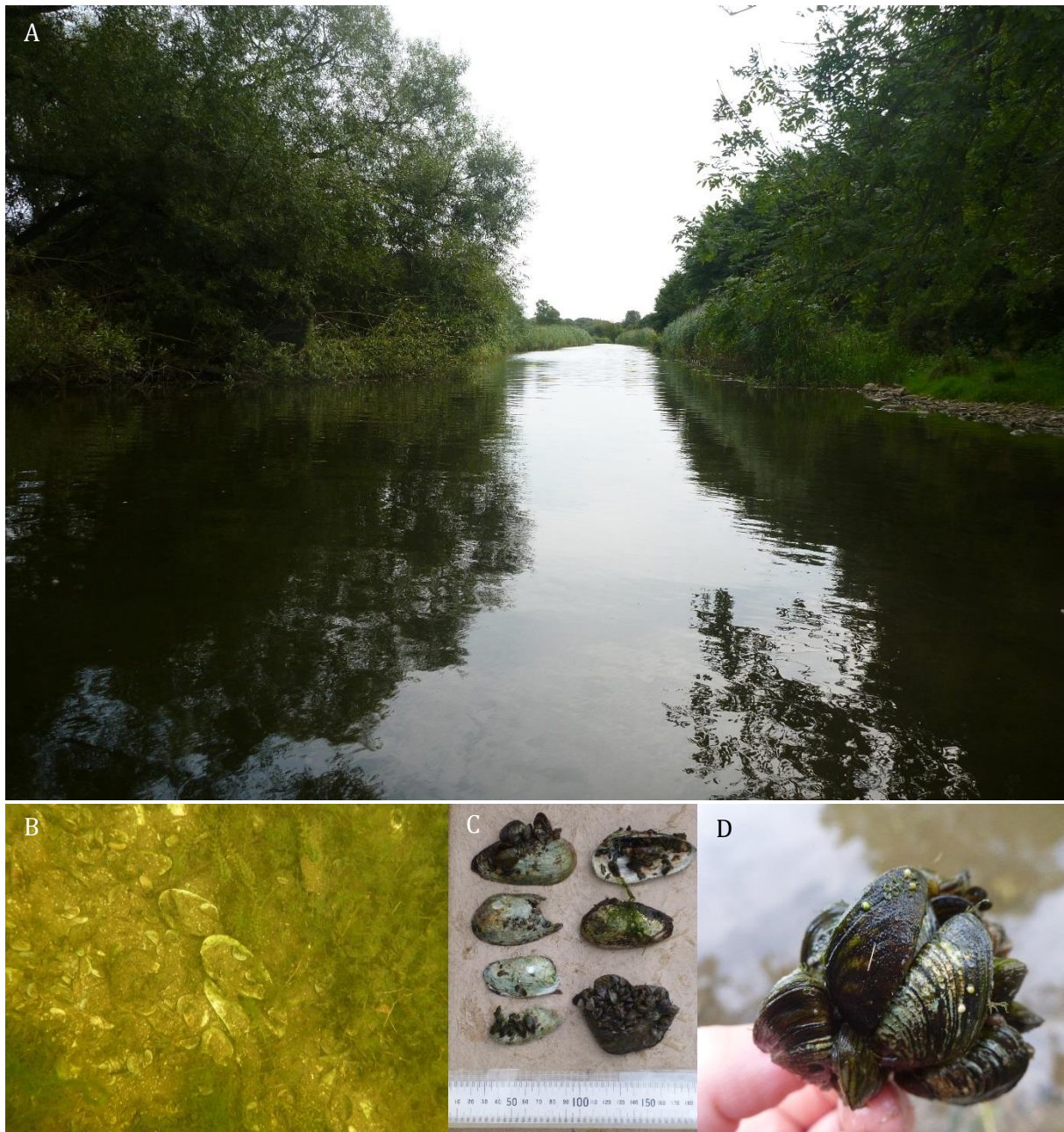


Figure 14. The River Suså at Stridmøllehus – UC9 –in upstream direction (A); stream bottom at UC9 covered with mussel shells (*U. tumidus* and *D. polymorpha*) and the macrophyte *Elodea Canadensis* (B); mussel shells of *Unio tumidus* covered with the invasive mussel species *Dreissena polymorpha* (C); conglomerate of *D. polymorphata* (D) .



Figure 15. The River Suså at Holløse Mølle – UC10 –in upstream direction (A); shell aggregation of the freshwater and marine mussels *D. polymorpha* and *Mytilus spp.* (B); aggregation of the invasive species *Dreissena polymorpha* (C); shell aggregation of *D. polymorpha* at UC10 (D).

Specific investigation

The specific mussel investigation was conducted at the survey site UC0, where most living *U. crassus* were found during the overview investigation. On the 20 m stretch screened thoroughly for mussels, a total of 325 living mussels was found, with an average of 16 mussels per investigated meter (Fig. 16, Appendix II).

Among three whole shells and eight shell valves of *U. crassus*, one living individual with a length of > 30 mm was found (Fig. 16D, Appendix II). No further living *U. crassus* was detected during an extended search 20 m up- and downstream of the survey site for 30 minutes. Hence, extrapolating the results from the specific investigation to a 100 m river stretch results in an intermediate abundance of 5-20 individuals/100m of living *U. crassus* and a high shell abundance of > 20 individuals/100m.



Figure 16. Picture of the riverbed at UC0 (A); living mussel and shells collected during the specific investigation for species identification (B,C); living individual of *U. crassus* found during the specific investigation (D).

Additional screening

An additional search for *U. crassus* upstream of the survey sites UC0 to UC8 in the Upper Suså did not result in further detection of living *U. crassus* individuals. Shell findings occurred at three locations, which is downstream of the survey site UC0, between UC3 and UC4, and between UC4 and UC5. This information is summarized in table 2, together with findings of other mussel species and habitat parameters noted for the locations investigated. Pictures of selected locations are provided below.

Table 1. Summary of the additional screening upstream of survey sites UC0 to UC8 in the Upper Suså. The presence of UC, *Unio crassus*.

	Location in river	Coordinates WGS84 (N, E)	UC shell abundance	Comments
Flow direction ↓	Bridge at Broksøvej	55.306513, 11.837275	absent (0)	Presence of living <i>U. tumidus</i> , <i>A. cygnea</i> , and <i>A. anatina</i> . Intermediate macrophyte cover and low algae cover.
		55.32655, 11.83566	absent (0)	Living mussels (<i>U. tumidus</i>) particularly under trees. Very turbid water.
	Upstream of tributary	55.32720, 11.84307	absent (0)	Presence of living <i>U. tumidus</i> exclusively under tree. Soft sediment in which small mussels burry.
	Downstream of tributary	55.327734, 11.84574	absent (0)	High algae cover and presence of <i>Elodea spp.</i> downstream of tributary. Low mussel shell cover (<i>U. tumidus</i>). Presence of living mussels particularly under trees.
	Between tributaries	55.32792, 11.84846	absent (0)	Less algae cover. No living mussels but shells of <i>U. tumidus</i> .
	Søbaek tributary	55.32841, 11.84927	NA	Turbid water carrying sediment loads from the tributary mixing with more transparent water from the River Suså. High algae cover.
	Downstream of tributary	55.32900, 11.84938	absent (0)	River more narrow; high macrophyte cover; fewer shells and living mussels (<i>U. tumidus</i>), high algae presence.
	At bridge near Teestrup	55.3942, 11.84894	absent (0)	Only few living individuals of <i>U. tumidus</i> present.
		55.33046, 11.84846	absent (0)	High presence of mussel shells (mostly <i>U. tumidus</i>), together with heterogen sediment and less algae cover.
		55.33163, 11.84685	NA	High algae and macrophyte cover on a distinct area.
	Between UC0 and coordinates	55.33163, 11.84685	low (1-5/100m)	High mussel presence (mostly <i>U. tumidus</i>) and reduced cover of macrophytes
	Between UC0-1	55.33508, 11.85055	NA	Different sediment composition (more fine sediments) and higher algae presence after tributary, possible deriving from increased nutrient load.
	Between UC3-4	55.34865, 11.87421	low (1-5/100m)	
	Between UC4-5	55.35548, 11.86141	low (1-5/100m)	



Figure 17. The River Suså at the bridge of Broksøvej looking in upstream (A) and downstream (B) direction; living individuals of *Unio tumidus* and *Anodonta anatina* (C).



Figure 18. The River Suså at the tributary west of Henriettelund where clear water is mixing with the turbid water from the tributary.

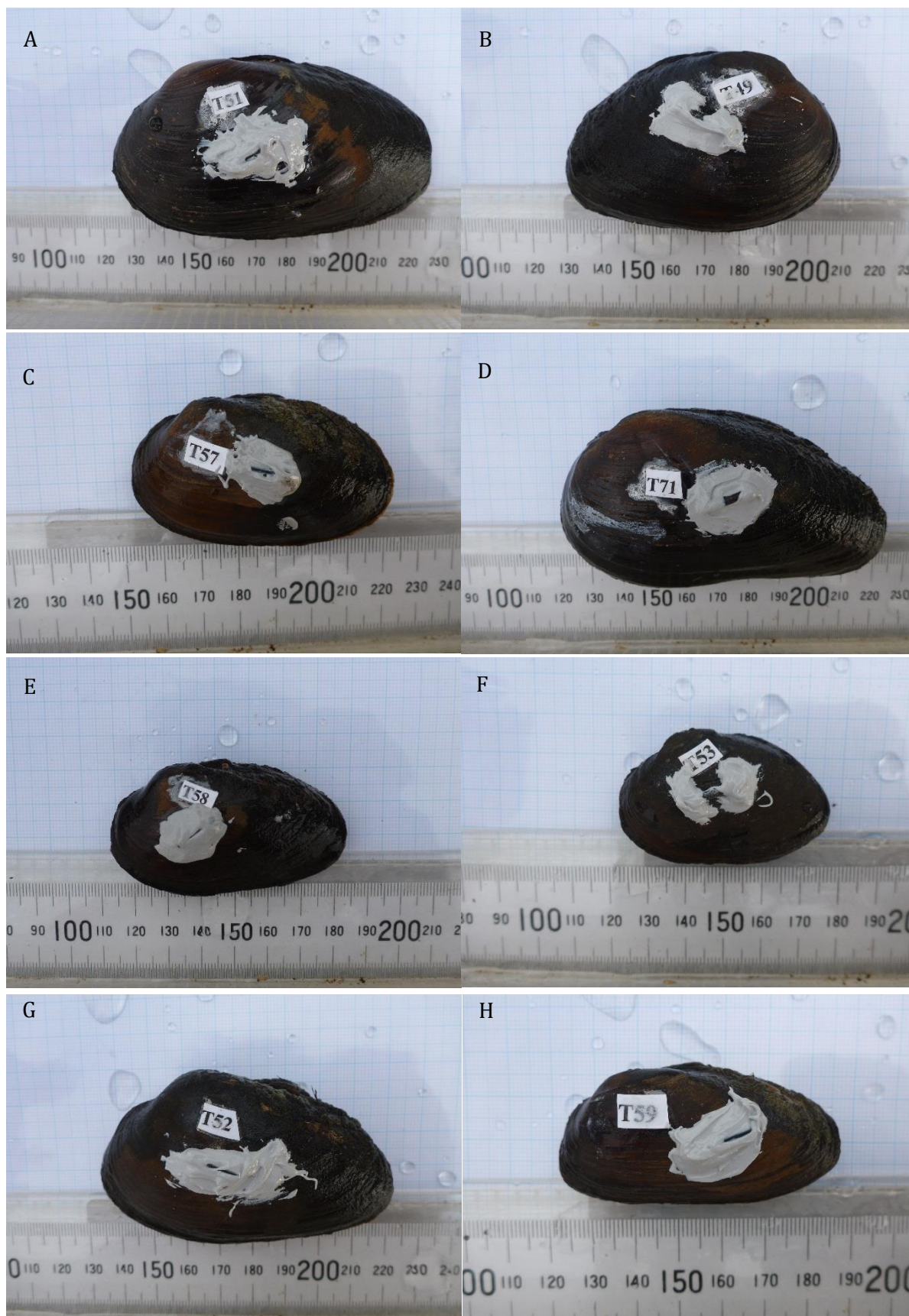


Figure 19. Examples of PIT tagged mussel individual of *Unio crassus* (A-D), *U. tumidus* (E-G) and *U. pictorum* (H) found in the River Suså.

Tagging of mussels and DNA results

A total of 23 mussels, including a variety of mussel species were tagged with a PIT and an individual number (Fig. 19, table 3). Soft mussel tissue was sampled for species identification using molecular techniques. Six of the mussel individuals were genetically identified as *U. crassus* (e.g. Fig 19A-D). Sixteen mussel individuals were identified as *U. tumidus* (e.g. Fig. 19 E-G) and one individual was identified as *U. pictorum* (Fig. 19H).

Table 2. Summary of mussel identifiers and shell size of tagged mussels. Ind.no., individual tag number; PIT no., PIT number.

River	Location	Date	Ind. no.	Mussel species (DNA results)	PIT no.	Length (mm)	Width (mm)	Height (mm)	Comments
Suså	UC0	2017.08.22	T49	<i>U. crassus</i>	226000785358	67	30	43	
Suså	UC0	2017.08.22	T50	<i>U. crassus</i>	226000785329	95	37	52	
Suså	UC0	2017.08.22	T51	<i>U. crassus</i>	226000733568	86	36	49	
Suså	±UC0	2017.08.26	T67	<i>U. crassus</i>	226000733535	83	33	47	
Suså	±UC0	2017.08.26	T71	<i>U. crassus</i>	226000733522	87	33	49	
Suså	UC8	2017.08.24	T57	<i>U. crassus</i>	226000809113	62	22	36	
Suså	UC8	2017.08.24	T59	<i>U. pictorum</i>	226000785391	57	21	27	
Suså	±UC0	2017.08.26	T64	<i>U. tumidus</i>	226000785379	56	22	36	
Suså	±UC0	2017.08.26	T65	<i>U. tumidus</i>	226000733573	70	27	38	
Suså	±UC0	2017.08.26	T66	<i>U. tumidus</i>	226000785317	40	18	25	
Suså	±UC0	2017.08.26	T70	<i>U. tumidus</i>	226000733590	50	19	28	
Suså	UC0	2017.08.22	T52	<i>U. tumidus</i>	226000733537	76	32	45	
Suså	UC0	2017.08.22	T53	<i>U. tumidus</i>	226999733569	49	21	30	not in good condition
Suså	UC0	2017.08.22	T54	<i>U. tumidus</i>	226000733597	63	26	35	
Suså	UC0	2017.08.22	T55	<i>U. tumidus</i>	226000733591	50	21	29	
Suså	UC0	2017.08.22	T56	<i>U. tumidus</i>	226000785325	77	30	46	used as reference
Suså	UC0	2017.08.26	T68	<i>U. tumidus</i>	226000785377	50	19	27	
Suså	UC0	2017.08.26	T69	<i>U. tumidus</i>	226000808982	44	17	25	
Suså	UC8	2017.08.24	T58	<i>U. tumidus</i>	226000529770	64	23	36	
Suså	UC8	2017.08.24	T60	<i>U. tumidus</i>	226000785328	55	20	30	
Suså	UC8	2017.08.24	T61	<i>U. tumidus</i>	226000733510	78	27	41	
Suså	UC8	2017.08.24	T62	<i>U. tumidus</i>	226000138353	55	21	31	
Suså	UC8	2017.08.24	T63	<i>U. tumidus</i>	226000733528	44	16	25	

Concluding summary

Historical data about one living individual of *U. crassus* in the River Suså could be confirmed by the recent mussel investigation conducted in August 2017. A total of 6 living individuals was detected in the Upper Suså, with one individual found at the survey site near Vrangstrup and five individuals found at the survey site near Assendrup. According to the categorization by Wiberg-Larsen (2015), these mussel occurrences represent 'low' abundance of *U. crassus* (1-5 individuals per 100 meter river length). However, deep waters limited the recent mussel search for *U. crassus* carried out by wading in up and downstream direction of the survey site near Vrangstrup. Hence, further individuals of *U. crassus* may be detected when diving is applied.

U. crassus found north of the small bridge near Assendrup may represent individuals of the oldest population in the Upper Suså, as dredging has never been conducted at this river stretch surrounded by forest which limiting the access for excavators. This is supported by the fact that

very large individuals of *U. crassus* were found. Moreover, an old and dense population of the swollen river mussel *U. tumidus*, the generally dominant mussel species in the Upper Suså, is present near Assendrup. Further individuals of *U. crassus* may exist along with the dense population of *U. tumidus*, albeit the extensive search, which included manual digging for mussels.

Shells of *U. crassus* were detected at low (1-5 shells per 100 meter river length) to high (> 20 shells per 100 meter river length, near Assendrup) abundances in the Upper Suså. Often, shells were highly corroded or overgrown by algae. This can hint at the death of the mussel far back in the past or at high levels of erosion in the river. Due to the generally low numbers of living *U. crassus* and of shells found in the Upper Suså, very few if any living *U. crassus* are expected to remain undetected.

In the Lower Suså, no living individuals of *U. crassus* and shells were recorded. It therefore remains unclear whether *U. crassus* still exists in the Lower Suså. Historical data hints at a former presence of this mussel species in the Lower Suså. However, the probability of a present existence is low as an extensive invasion of *D. polymorpha* occurs in the Lower Suså. *Dreissena polymorpha* is able to attach to all kinds of substrates, such as native mussel species, and hereby outcompete them for food (Strayer et al. 2004). During the recent mussel search, shells of native mussels species other than *U. crassus* were found colonized by *D. polymorpha*. No living mussels were found. Thus, living individuals of native mussels, if still present in the Lower Suså, are difficult to detect as syphons are covered by aggregations of *D. polymorpha*. To avoid further dispersal of *D. polymorpha*, particularly from the Lower to the Upper Suså, where colonization of the invasive species implies a high threat for native mussels, no exchange of water or equipment, including boats, waders, or fishing rods, should take place prior to thorough disinfection. This is because *D. polymorpha* has a reproduction mode similar to marine blue mussels - over free-living veliger larvae that develop independently from fish. These larvae can attach to all kind of surfaces, thus spread easily.

Concluding, it can be said, that *U. crassus* still exists in the Upper Suså. Due to the low species abundance, the reproduction potential may however be limited. Further mussel investigations in the Upper Suså may increase the number of individuals. However, the possibility of successful reproduction of *U. crassus* in Suså seems low. Conservation measures of *U. crassus* and its affiliated host fish species is highly recommended to preserve extinction of this species in the river.

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Appendix

Appendix I. Summary of historical and present records of shells and living individuals of *Unio crassus* in the River Suså and Torpe Kanal.

River	Location	Inventory		Record of <i>Unio crassus</i>		Responsible	Further information
		Date	No.	Living	Shells		
Suså	Stridmøllehus	24-08-2017	38	absent (0)	absent (0)	KAU	
	Syd for Almtofte	23-08-2017	36	absent (0)	intermediate (5-20/100m)	KAU	
	Lunden Skov	23-08-2017	35	absent (0)	few (1-5/100m)	KAU	
	Øst for Lunden Skov	23-08-2017	34	absent (0)	few (1-5/100m)	KAU	
	Granskiftegaard	22-08-2017	33	absent (0)	few (1-5/100m)	KAU	
	North of Henriettelund	26-08-2017	31	absent (0)	few (1-5/100m)	KAU	
	Næsby Bro	15-06-2015	2	0	few (1-5/100m)	Miljøstyrelsen	
		15-06-2015	3	0	few (1-5/100m)	Miljøstyrelsen	
	100 meter NS Ringsted Å	11-06-2015	4				
	Assendrup	22-08-2017	30	6	intermediate (5-20/100m)	KAU	
		29-05-2007	27	Ingen	few (1-5/100m)	Miljøstyrelsen	
		24-08-2017	39	absent (0)	absent (0)	KAU	
	Holløse Bro	30-05-2007	23	Ingen	many (>20/100m)	Miljøstyrelsen	
		09-06-2015	7	0	absent (0)	Miljøstyrelsen	
	Kanosted Skelby/ Gunderslevholm	09-06-2015	8	0	absent (0)	Miljøstyrelsen	
	Møllebro ved Eskildstrup	23-08-2017	34	absent (0)	few (1-5/100m)	KAU	
	Mølle	29-05-2007	30	Ingen	few (1-5/100m)	Miljøstyrelsen	
	nedstrøms Lilleå (Novana st.)	25-06-2007	21	Ingen	many (>20/100m)	Miljøstyrelsen	
		16-06-2015	1	0	few (1-5/100m)	Miljøstyrelsen	
	Ny Møllebro	29-06-2007	20	Ingen	intermediate (5-20/100m)	Miljøstyrelsen	
	Teestrup Bro	22-08-2017	32	absent (0)	absent (0)	KAU	
		11-06-2008	19	Ingen	intermediate (5-20/100m)	Miljøstyrelsen	
	Vetterslev	11-06-2015	5	0	few (1-5/100m)	Miljøstyrelsen	
	Vetterslev, Gl. Næstvedvej.	29-05-2007	29	Ingen	absent (0)	Miljøstyrelsen	
		24-08-2017	36	2	few (1-5/100m)	KAU	
Torpe Kanal	Vrangstrup	10-06-2015	6	1	few (1-5/100m)	Miljøstyrelsen	http://naturereport.udv.miljoportal.dk/735061
		29-05-2007	28	Ingen	few (1-5/100m)	Miljøstyrelsen	
	Holmen	21-09-2009	16	0	many (>20/100m)	Miljøstyrelsen	
		30-05-2007	25	Ingen	many (>20/100m)	Miljøstyrelsen	
	Jeppebro	21-09-2009	15	Ingen	absent (0)	Miljøstyrelsen	
	NS bro ved CC Bruun	08-06-2015	9	Ingen	few (1-5/100m)	Miljøstyrelsen	
	opstrøms Ravnstrup Skov	21-09-2009	14	1		Miljøstyrelsen	http://naturereport.udv.miljoportal.dk/497967
	OS jernbanebro	24-08-2017	40	6	intermediate (5-20/100m)	KAU	Technical report: Mussel inventory in Torpe Kanal
		27-05-2015	11	3	many (>20/100m)	Miljøstyrelsen	http://naturereport.udv.miljoportal.dk/735061
	Ravnstrup Skov - langt OS	08-06-2015	10	Ingen	intermediate (5-20/100m)	Miljøstyrelsen	
	Jernbane	30-05-2007	24	Ingen	intermediate (5-20/100m)	Miljøstyrelsen	
	Regerup						
		27-05-2015	12	2	many (>20/100m)	Miljøstyrelsen	http://naturereport.udv.miljoportal.dk/735061
	Skullerup Bro	21-09-2009	13	6		Miljøstyrelsen	http://naturereport.udv.miljoportal.dk/497995
		11-06-2008	18	3	many (>20/100m)	Miljøstyrelsen	http://naturereport.udv.miljoportal.dk/405168
	Tingbro	21-09-2009	17	0	many (>20/100m)	Miljøstyrelsen	
	vej Regerup-Nødholm	30-05-2007	22	Ingen	few (1-5/100m)	Miljøstyrelsen	
	vej til Bavelse	30-05-2007	26	Ingen	many (>20/100m)	Miljøstyrelsen	

Appendix II. Number of mussels findings (alive, shells and half shells) of *Unio crassus*, *U. tumidus*, *Anodonta anatina* and *A. cygnea* for each investigation step during the specific mussel investigation at the survey site UC0. The average depth (m) and width (m) of the river is given for each investigation step and the total number of mussels alive.

Step	Width	Depth	<i>U. crassus</i>			<i>U. tumidus</i>			<i>A. anatina</i>			<i>A. cygnea</i>			TOTAL.
(m)	(m)	(m)	alive	shell	half shell	alive	shell	half shell	alive	shell	half shell	alive	shell	half shell	no. alive
1	5.6	34	0	0	0	16	20	3	0	0	0	0	2	3	16
2	5.5	42	0	0	0	26	39	10	0	2	0	0	11	5	26
3	5.6	45	0	0	0	23	31	6	0	1	0	0	9	1	23
4	5.6	42	0	0	0	15	28	0	0	2	0	0	1	7	15
5	5.6	45	0	0	3	12	35	4	0	0	0	0	3	9	12
6	5.5	43	0	1	0	7	9	2	0	0	0	0	0	1	7
7	5.4	46	1	0	0	5	18	2	0	0	0	0	1	1	6
8	5.6	42	0	1	0	4	16	0	0	0	1	0	2	1	4
9	5.7	46	0	0	0	9	31	1	0	3	0	0	1	3	9
10	5.6	68	0	0	2	10	23	2	0	1	1	0	0	1	10
11	5.4	74	0	0	0	14	28	2	0	0	0	0	4	3	14
12	5.3	65	0	0	2	13	36	0	0	0	0	0	0	5	13
13	5.2	48	0	0	0	17	NA	NA	0	NA	NA	0	NA	NA	17
14	5.2	48	0	0	1	43	NA	NA	0	NA	NA	0	NA	NA	43
15	5.1	53	0	0	0	21	NA	NA	0	NA	NA	1	NA	NA	22
16	4.9	50	0	0	0	45	NA	NA	0	NA	NA	0	NA	NA	45
17	5.1	47	0	1	0	16	NA	NA	0	NA	NA	0	NA	NA	16
18	5.0	45	0	0	0	7	NA	NA	0	NA	NA	0	NA	NA	7
19	5.0	45	0	0	0	11	NA	NA	0	NA	NA	0	NA	NA	11
20	5.1	45	0	0	0	9	NA	NA	0	NA	NA	0	NA	NA	9
															325

Appendix III. Summary of data from the overview mussel investigation in the Upper Suså. AA, *Anodonta anatina*; AC, *Anodonta cygnea*; DP, *Dreissena polymorpha*; UC, *Unio crassus*; UT, *Unio tumidus*. Decimals are separated by commas.

River	Upper Suså						
Locality	UC0	UC1	UC2	UC3	UC4	UC5	
	Assendrupvej	North of Henriettelund	Teestrup Bro	Granskiftegaard	Eskildstrup Møllebro	Øst for Lunden Skov	
GPS coordinates (WGS84)	N: 55.33320	55.33314	55.33444	55.34433	55.35054	55.35804	
	E: 11.84685	11.86280	11.87769	11.87985	11.86596	11.85326	
Investigation date and time	date 2017-08-22	2018-08-26	2017-08-22	2017-08-22	2017-08-23	2017-08-23	
	start time 10:30	10:30	16:30	18:00	11:45	14:30	
Method	end time 14:30	11:30	17:30	19:00	13:00	15:30	
	time spent (h) 4	1	1	1	1.25	1	
River stretch	length (m) 100	wading	wading	wading	wading	wading	
	av. width (m) 5.6	100	100	75	100	100	
	av. depth (m) 0.7	4.6	4.5	5.4	5.2	4.1	
	max. depth (m) 0.8	0.4	0.4	0.7	0.7	0.4	
Unio crassus	no. living 6	0.5	0.7	0.8	0.8	0.5	
	age older ≥ 30mm	0	0	0	0	0	
Presence of other living mussels species	density living low (1-5/100m)	NA	NA	NA	NA	NA	
	density shells high (>20/100m)	absent (0)	absent (0)	absent (0)	absent (0)	absent (0)	
	AA absent(0)	low (1-5/100m)	absent (0)	low (1-5/100m)	low (1-5/100m)	low (1-5/100m)	
	AC absent(0)	absent(0)	absent (0)	absent (0)	absent (0)	absent (0)	
	UP low (1-5/100m)	absent(0)	absent (0)	absent (0)	absent (0)	absent (0)	
	UT high (>20/100m)	? intermediate (5-20/100m)	? intermediate (5-20/100m)	? intermediate (5-20/100m)	? intermediate (5-20/100m)	? intermediate (5-20/100m)	
Shell presence of other mussel species	DP absent(0)	intermediate (5-20/100m)	absent (0)	absent (0)	low (1-5/100m)	low (1-5/100m)	
	AA low (1-5/100m)	absent(0)	absent (0)	absent (0)	absent (0)	absent (0)	
	AC low (1-5/100m)	absent (0)	absent (0)	absent (0)	absent (0)	absent (0)	
	UP low (1-5/100m)	low (1-5/100m)	low (1-5/100m)	low (1-5/100m)	low (1-5/100m)	low (1-5/100m)	
Sediment	UT high (>20/100m)	? intermediate (5-20/100m)	? intermediate (5-20/100m)	? intermediate (5-20/100m)	low (1-5/100m)	? intermediate (5-20/100m)	
	DP absent(0)	high (>20/100m)	low (1-5/100m)	intermediate (5-20/100m)	intermediate (5-20/100m)	intermediate (5-20/100m)	
	heterogeneity intermediate (2)	absent(0)	absent (0)	absent (0)	absent (0)	absent (0)	
	sand (%) 10	intermediate (2)	absent (0)	absent (0)	absent (0)	intermediate (2)	
River regulation	granules + pebbles (%) 60	intermediate (2)	little (1)	little (1)	little (1)	intermediate (2)	
	cobbles & stones (%) 30	20	70	20	10	10	
		50	20	60	70	50	
		30	10	20	20	40	
	no artificial impact (0)	no artificial impact (0)	1 (bridge; dock from landowner)	1 (construction waste; bridge)	1 (bridge)	no artificial impact (0)	
Macrophyte vegetation	Sparganium spp.	Sparganium spp.; Lemna spp.; Phragmites australis; Callitriche spp.	Sparganium spp.; Potamogeton spp.	Potamogeton spp.	Sparganium spp.; Lemna spp.; Elodea canadensis	Potamogeton spp.; Sparganium spp.	
	species	oak	alder, willow	spruce	maple, alder, birch	willow	
Riparian	(%) 90	40	30	30	2	2	
Shallow							
Fish presence	species	Gymnocephalus cernuus; Leuciscus cephalus	present (1)	present (1)	present (1)	absent (0)	
Comments		Morphological similarities between U. crassus and U. tumidus; mussels most aggregated in the middlecourse; metal in the water giving mussels a copper rust; low sediment penetration resistance					
			Iron sticks in the water; lots of algae and macrophytes	Partly covered with algae; substrate was partly artificial; bridge; clear water; clogged sediment	Up to 90 % macrophyte coverage; underneath fine sediment	Living Unio tumidus were very small	

Table continues on next page.

Appendix III. Table continued

River	Upper Suså			Lower Suså		
Locality	UC6	UC7	UC8	UC9	UC10	
	Lunden Skov	Syd for Almtofte	Vrangstrup	Stridmøllehus	Holløse Mølle	
GPS coordinates (WGS84)	55.359618 11.844678	55.367314 11.817727		55.401121 11.724665	55.324896 11.659563	55.299911 11.689625
Investigation date and time	2017-08-23 16:10 17:00	2017-08-23 18:00 19:00		2017-08-24 09:00 11:00	2017-08-24 12:45 13:45	2017-08-24 14:15 15:30
Method	0.85 wading	1 wading		2 wading	1 wading and by boat	1.25 wading
River stretch	length (m) av. width (m) av. depth (m) max. depth (m)	116 5.6 0.2 0.7	100 5.1 0.7 1.4	50 7.3 0.9 1.6	100 13.9 0.9 >1.5	100 19.8 0.4 >1.5
Unio crassus	no. living age density living density shells	0 NA absent (0) low (1-5/100m)	0 NA absent (0) intermediate (5-20/100m)	2 older ≥ 30mm low (1-5/100m) low (1-5/100m)	0 NA absent (0) absent (0)	0 NA absent (0) absent (0)
Presence of other living mussels	AA AC UP UT DP	absent (0) absent (0) ? low (1-5/100m) absent (0)	absent (0) absent (0) ? absent (0) absent (0)	absent (0) absent (0) ? intermediate (5-20/100m) absent (0)	absent (0) absent (0) ? absent (0) high (>20/100m)	absent (0) absent (0) ? absent (0) high (>20/100m)
Shell presence of other mussel species	AA AC UP UT DP	absent (0) low (1-5/100m) ? low (1-5/100m) absent (0)	low (1-5/100m) low (1-5/100m) ? low (1-5/100m) absent (0)	absent (0) absent (0) ? intermediate (5-20/100m) absent (0)	absent (0) absent (0) ? low (1-5/100m) high (>20/100m)	absent (0) low (1-5/100m) absent (0) low (1-5/100m) high (>20/100m)
Sediment	heterogeneity sand (%) granules + pebbles (%) cobble & stones (%)	little (1) 10 70 20	little (1) 20 40 40	intermediate (2) 0 60 40	little (1) 70 20 10	intermediate (2) 10 60 30
River regulation		no artificial impact (0)	no artificial impact (0)	1 (bridge)	no artificial impact (0)	3 (bridge; artificial sediment; dam)
Macrophyte vegetation		Sparganium spp.; Lemna spp.	Sparganium spp.; Nuphar lutea (submerged)	Sparganium spp.; Phragmites australis	Nuphar lutea (submerged); Phragmites australis; Elodea spp.	Potamogeton spp.; Nuphar lutea; Myriophyllum spp.
Riparian Shadlow	species (%)	willow, maple 35	willow 5	no trees 1	willow 5	willow 1
Fish presence	species	present (1)	absent (0)	present (1)	present (1)	present (1)
Comments		Partly very shallow		Lots of algae and fine sediment	Zebra mussel sits on all surfaces available, incl. mussel shells. High presence of algae and fine sediment.	Marine bivalve and gastropoda shells in the water, together with artificial sediment. Zebra mussel dominant species.



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