

Results from a small mussel inventory in Suså and Torpe Kanal

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Background

On April 30 and May 16-17, a mussel inventory was conducted in the rivers Suså and Torpe Kanal, located on Sjælland – Denmark, in order to follow up previously (August, 2017) tagged individuals of the thick-shelled river mussel *Unio crassus* (Schneider and Zülsdorff, 2017, 2018) as well as to search for further individuals in both rivers. Knowledge on the status (existence, survival, reproduction and genetic background) of *U. crassus* in both rivers is of overall importance for mussel conservation efforts, such as the re-introduction/population enhancement of this endangered mussel species, conducted in the European LIFE project UC LIFE Denmark.

Methods

On April 30, the monitoring started with the recapture of previously tagged *U. crassus* at two river stretches located in the River Suså, nearby the road Assendrupvej (locationUCo) and in the River Torpe Kanal, nearby the railway bridge at the village Skullerup (location 6), respectively (figure 1, table 1). A total number of six *U. crassus* was tagged at both locations in June and August 2017 (table 1, Schneider and Zülsdorff, 2017, 2018).

Monitoring was conducted by wading in the rivers and visual investigation of the riverbed using aquascopes. Detected mussel individuals were picked by hand, examined for individual PIT- (Passive Integrated Transponder) and number tags. Moreover, mussels were visually examined for gravidity according to the instructions of Bednarczuk (1986). Samples of mussel brood, if present were taken and visually inspected using a microscope binocular in order to evaluate the status of the brood, i.e. if egg fertilization has occurred and brood is developing to mussel larvae (glochidia).

After the recapture of tagged mussels on April 30, an additional search for *U. crassus* was conducted in the Torpe Kanal at location 6, on a 200 m-long stretch, for about one hour (table 1). The search was repeated on May 16, where the same river stretch was investigated and extended for about 300 m in upstream and 140 m in downstream direction (location 5).

The same day, a furthered search for mussels was conducted in the River Suså, nearby the village of Vrangstrup (UC8) where one individual of *U. crassus* was found during the 2017-monitoring and moved to location UCo (Schneider and Zülsdorff, 2017, 2018). On May 17, three new locations, “Naesby bro” (location 1), “Railwaybridge near Veterslev” (location 2) and “Sandby bro” (location 3) were investigated for mussels in the River Suså, (figure 1, table 1). All *U. crassus* found in Torpe Kanal were aggregated at location 6 and *U. crassus* found in the River Suså at location UCo, as done during previous mussel investigations (Schneider and Zülsdorff, 2017, 2018).

Mussels newly detected were species identified using morphological traits, measured in size (length, height and width; mm) and were tagged with an individual number tag. All mussels found during the present monitoring were DNA-sampled and samples were sent to the Swedish

Museum of Natural History for species identification using ITS-analyses according to Källersjö et al. (2005).

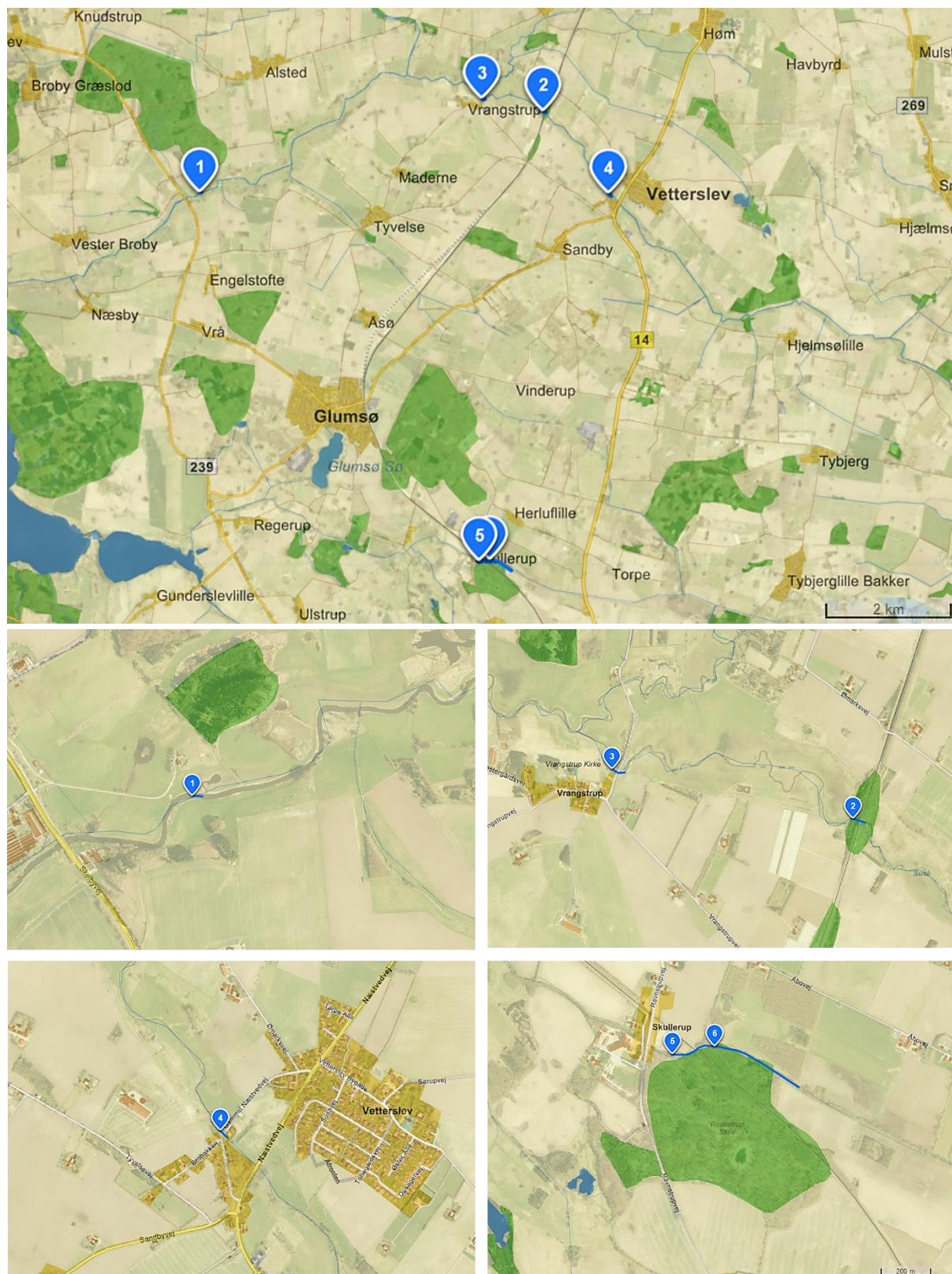


Figure 1. Monitoring locations in the rivers Suså (landmarks 1-4) and Torpe Kanal (landmarks 5-6). For location names see table 1. Source: <https://kartor.eniro.se/m/t1hlw>.

Table 1. Coordinates and length of monitoring locations in the rivers Suså and Torpe Kanal at which mussels were recaptured, monitored or aggregated during the present mussel survey.

River	Point	Location	Length (m)	Coordinates (WGS84: lat, lon)	
				Downstream	Uppstream
Suså	1	Næsby bro	4	55.387448, 11.652122	55.387442, 11.652447
Suså	2	Railwaybridge near Veterslev	30	55.399307, 11.740631	55.399181, 11.741453
Suså	3	UC8 - Vrangstrup	60	55.401195, 11.724727	55.401057, 11.725643
Suså	4	Sandby bro	50	55.38724, 11.757575	55.38695, 11.758055
Suså	UC0	Aggregation of tagged mussels nearby the road Assendrupvej	2	55.334278, 11.848908	55.33428, 11.848927
Torpe Kanal	5	Railwaybridge near Skallerup	550	55.333374, 11.724064	55.332147, 11.732401
Torpe Kanal	6	Aggregation of tagged mussels	2	55.333697, 11.726822	55.333697, 11.726897

Results and discussion

River Suså

During the investigations conducted in the River Suså in April and May 2019, two previously (August 2017) tagged individuals of *U. crassus* were found at location UC0 and one new additional individual was detected at location UC8 (table 2). None of the individuals was gravid. Whether this is due to the small population size of *U. crassus* in the River Suså is unclear but likely (Downing et al. 1993). At present five individuals of *U. crassus* are left at the location UC0 (table 2) as two of originally six tagged and aggregated mussels died during the summer 2018, and the newly detected individual from the present monitoring was translocated to the location. In order to evaluate the reproduction potential of the remaining mussel population in the River Suså it has yet to be clarified whether *U. crassus* is represented by (i) males only, (ii) females not reproducing or (iii) females reproducing at different timings than examined.

Future breeding of mussels from the River Suså for population enhancement is difficult and genetically questionable if no additional and gravid individuals are detected. Further mussel monitoring, particularly at location UC8, where an additional individual of *U. crassus* was found during the present monitoring could result in the detection of further individuals. Moreover, the detection of the remaining two individuals tagged with a Passive Integrated Transponder (PIT) at location UC0 using a portable antenna helps evaluating whether there are reproducing females among *U. crassus*. If no reproductive individuals are found, captive

breeding could be based on the intake of *U. crassus* from the River Suså to an aquaria laboratory before the start of the mussel reproduction season, i.e. before females start to produce eggs, and before males release their sperms to the free-flowing water. Improved food supply and water quality may enhance mussel condition, possibly triggering mussels to take part in reproduction. Moreover, mussel aggregation at defined water volumes in the lab may enable egg fertilization by sperms (Eybe et al., 2014). However, genetical effects in small founder populations should be considered (Hoftyzer et al., 2008).

Table 2. Overview of mussel individuals found in the River Suså (and PIT tagged) during investigations conducted between 2017 and 2019. NG indicates that the individual was not gravid, Dead that the individual was found dead and a cross (x) indicates that the mussel was found, but not examined for gravidity. Stars (*) indicate that the mussel species was confirmed by results from DNA-analysis.

Location		Date (month/year)			Species	ID	PIT no.	Mussel size (mm)		
Original	Moved to	08/17	04/19	05/19				Length	Width	Hight
UC0	UC0	x			<i>U. crassus</i> *	T49	226000785358	67	30	43
UC0	UC0	x	Dead		<i>U. crassus</i> *	T50	226000785329	95	37	52
UC0	UC0	x	NG		<i>U. crassus</i> *	T51	226000733568	86	36	49
±UC0	UC0	x	NG		<i>U. crassus</i> *	T67	226000733535	83	33	47
±UC0	UC0	x	Dead		<i>U. crassus</i> *	T71	226000733522	87	33	49
UC8	UC0	x			<i>U. crassus</i> *	T57	226000809113	62	22	36
UC8	UC0			NG	<i>U. crassus</i> *	S55	Not tagged	60	21	34
UC0	UC0	x			<i>U. tumidus</i> *	T52	226000733537	76	32	45
UC0	UC0	x			<i>U. tumidus</i> *	T53	226999733569	49	21	30
UC0	UC0	x		x	<i>U. tumidus</i> *	T54	226000733597	63	26	35
UC0	UC0	x		x	<i>U. tumidus</i> *	T55	226000733591	50	21	29
±UC0	UC0	x			<i>U. tumidus</i> *	T64	226000785379	56	22	36
±UC0	UC0	x		x	<i>U. tumidus</i> *	T65	226000733573	70	27	38
±UC0	UC0	x			<i>U. tumidus</i> *	T66	226000785317	40	18	25
±UC0	UC0	x			<i>U. tumidus</i> *	T70	226000733590	50	19	28
UC8	UC0	x		x	<i>U. tumidus</i> *	T58	226000529770	64	23	36
UC0	UC0	x			<i>U. tumidus</i> *	T56	226000785325	77	30	46
UC0	UC0	x			<i>U. tumidus</i> *	T68	226000785377	50	19	27
UC0	UC0	x		x	<i>U. tumidus</i> *	T69	226000808982	44	17	25
UC8	UC0	x			<i>U. pictorum</i> *	T59	226000785391	57	21	27
UC8	UC0	x		x	<i>U. tumidus</i> *	T60	226000785328	55	20	30
UC8	UC0	x			<i>U. tumidus</i> *	T61	226000733510	78	27	41
UC8	UC0	x		x	<i>U. tumidus</i> *	T62	226000138353	55	21	31
UC8	UC0	x			<i>U. tumidus</i> *	T63	226000733528	44	16	25

In the following sections, monitoring results are presented for every location investigated in the River Suså.

Location UCo – near Assendrup

The search for mussels in the River Suså nearby the road Assendrupvej (location UCo) in April 2019 resulted in the recapture of nine tagged mussel individuals (figure 2, table 2). According to DNA-results from previous DNA analyses (Schneider and Zülsdorff, 2018) two of the

individuals were *Unio crassus* (number tags: T67 and T51) and seven *U. tumidus* (Number tags: T54, T58, T55, T65, T62, T60 and T69). None of the *U. crassus* was gravid. The individuals of *Unio crassus* found were two of originally six tagged mussels, whereof two died during the summer 2018 (personal communication Sofia Mulla Kølmel, Næstved municipality).



Figure 2. Mussel individuals found at the location UC0 during the investigation in April 2019.

Location UC8 – Vrangstrup

At the location near Vranstrup one shell valve and one living individual of *U. crassus* (S55, genetically approved, figure 1 and 3, table 1 and 2) were found directly upstream of the bridge, where one individual of *U. crassus* was detected, during the 2017-mussel investigation (Schneider and Zülsdorff, 2018). The newly found mussel was not gravid, but smaller and probably younger than *U. crassus* found in the River Suså so far (table 2). Even if lower water levels compared to 2017 allowed for wading in the river further upstream of the bridge, no additional individuals of *U. crassus* were found. Whether an additional investigation for *U. crassus* during low flow conditions and at times with low abundance of algae and aquatic vegetation would result in further individuals at the location is unclear, can however be considered. Light conditions were optimal during the present monitoring.

Several individuals of *Unio tumidus*, *Unio pictorum* and *Anodonta anatina* were also detected at the location (figure 3c). These species occurred at densities similar to described in Schneider and Zülsdorff (2018).



Figure 3. Location UC8 (a) and bottom substratum at UC8 (b). Detected living mussels at UC8 represent *Unio tumidus* (c), *Anodonta anatina* (c) and *Unio crassus* (c,d).

Location 1 – Næsby bro

The location close to Næsby bro has not been investigated for mussels earlier. The location is deep and did not allow for wading in the River Suså at more than 4 m river length and 1-2 m width (figure 1, table 1). Nevertheless, six individuals of *Unio tumidus* and four of *Unio pictorum* were found, though none of *U. crassus* (figure 4a). Lots of fine material, but also gravel and stones represented the bottom substratum (figure 4b). A comprehensive mussel monitoring would require diving at the location.



Figure 4. Mussel individuals found at location 1 (a). Vegetation and bottom substratum at location 1 (b).

Location 2 – Railwaybridge near Vetterslev

As location 1, this location has not been investigated for mussels earlier. The river just downstream of the bridge is difficult to wade through because of fine and soft sediment fractions (organic material and sand) and a depth of 1-2 m. Closer to the bridge the river becomes shallower and the bottom substratum stonier. Large areas of the bottom were covered with mussel shells, mostly *U. tumidus* and *U. pictorum*. Between the shells living individuals of different size were found of these species, however not of *U. crassus* (figure 5). Based on the present monitoring, reasons behind the high abundance of mussel shells are difficult to identify but may be related to nutrient runoffs from agriculture or old mussel populations that may accumulate many shells over time if little erosion occurs.

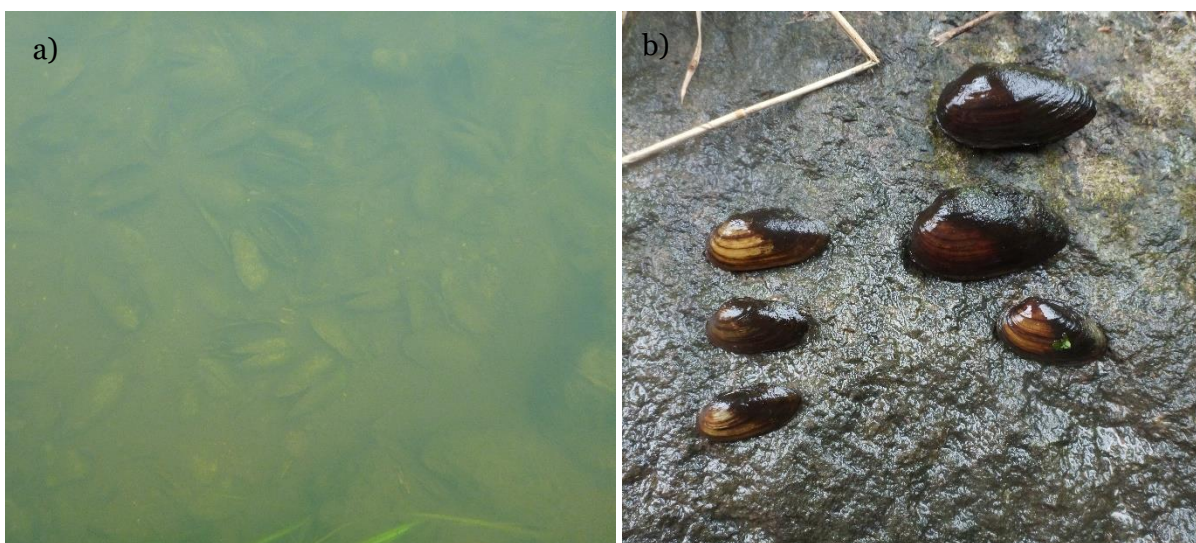


Figure 5. Shell findings (a) and living mussel individuals at location 2 in the River Suså.

Torpe Kanal

The search for mussels in the River Torpe Kanal in April 2019 resulted in the detection of four tagged individuals (Number tags: T2, T4, T5 and T12) and two additional individuals of *U. crassus* (T72 and T73; genetically approved; figure 6, table 3). In May, five previously tagged *U. crassus* were recaptured and six new individuals of *U. crassus* found (T74-T79; genetically approved; table 3). The additional mussels had a shell size similar to the mussels found during the mussel investigation conducted in 2017 (Schneider and Zülsdorff, 2017; see table 3). The shell size reached from 52 to 79 mm (figure 7) with an average of 66,7 mm. The currently known population size of *U. crassus* in Torpe Kanal is 13 individuals. These individuals were found on a 0,5 km long river stretch, which is why further individuals are assumed to occur in Torpe Kanal. Since the first monitoring in 2017, one tagged individual died. All *U. crassus* found in Torpe Kanal are aggregated at location 6 (figure 8).



Figure 6. Mussel individuals (*Unio crassus*) found during the investigations in the River Torpe Kanal in April and May 2019. Individuals with darker (overgrown) tags were tagged during a previous monitoring in 2017. Individuals with lighter tags were newly tagged.

One of the previously tagged (Tf2) and two of the additionally detected mussels (T73 and T78) were gravid. Mussel Tf2 was gravid already in 2017, indicating that this individual takes part in reproduction on a regular basis. Examination of brood samples showed however that eggs have not been developed to glochidia yet (figure 9a). Hence, it is unclear if egg fertilization took place and if eggs are developing to glochidia at a later stage. Shapes of glochidia were found in the brood sample of mussel T73 (figure 9b), which is why successful egg fertilization may have occurred. Concluding it can be said that an exceeding, though relatively time intense mussel monitoring in the River Torpe Kanal could result in the detection of additional mussel individuals and increases the potential of successful reproduction of aggregated mussels in the field. Captive breeding of aggregated mussels should consider genetic effects as elaborated in the management plan (Action: A1) for UC LIFE Denmark (Schneider and Österling, 2017).

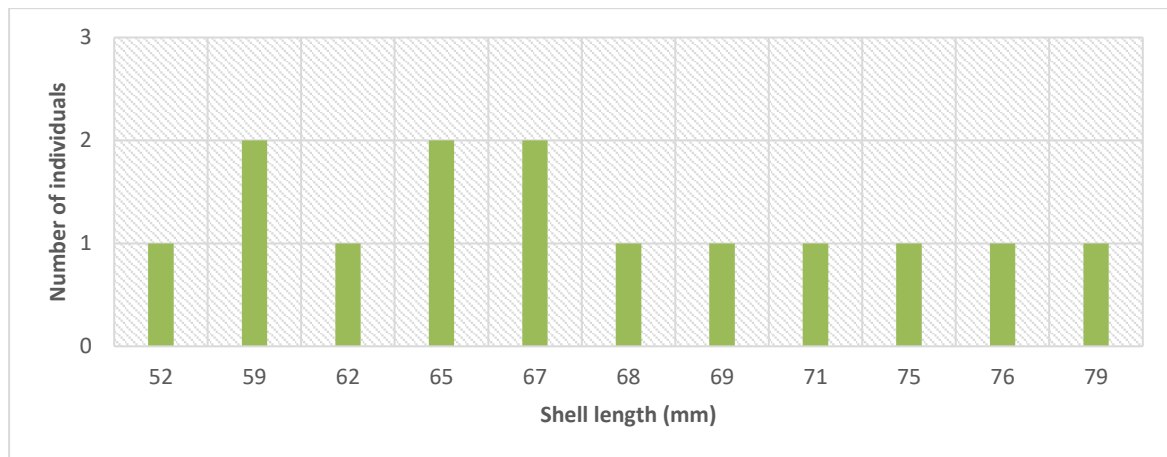


Figure 7. Size distribution of *Unio crassus* in the River Torpe Kanal.



Figure 8. Location in the River Torpe Kanal where mussels were aggregated after the monitoring (a) and upstream part of the river stretch investigated in Torpe Kanal (b).

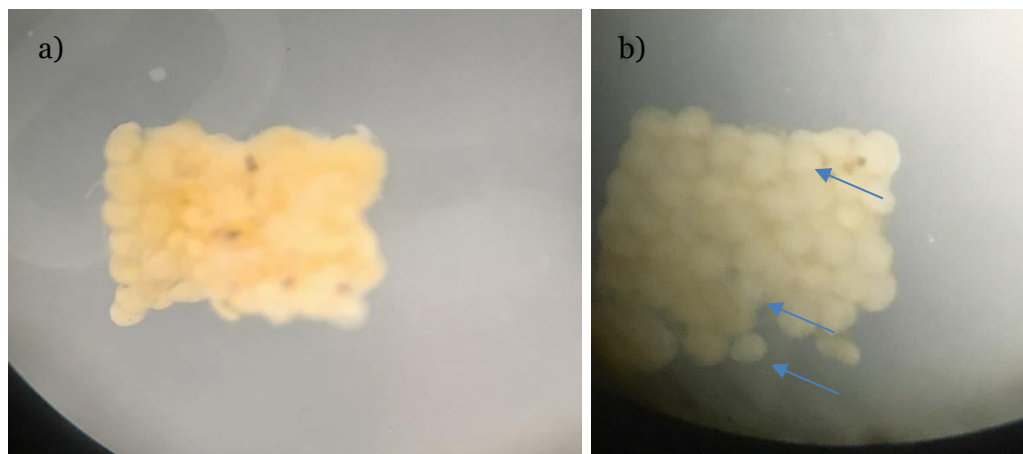


Figure 9. Brood samples from mussels Tf2 (a) and T73 (b) in the River Torpe Kanal. Blue arrows indicate eggs possibly developing to glochidia.

Table 3. Overview of mussel individuals found (and PIT tagged) in the River Torpe Kanal during investigations conducted between 2017 and 2019. NG indicates that the individual was not gravid, N that the individual was gravid, Dead that the individual was found dead, and a cross (x) indicates that the mussel was found, but not examined for gravidity. Stars (*) indicate that the mussel species was confirmed by results from DNA-analysis.

Location		Date (month/year)			Species	ID	PIT no.	Mussel size (mm)		
Original	Moved to	06/17	04/19	05/19				Length	Width	Hight
5	6	NG			<i>U. crassus</i>	T1	22600808974	62	23	37
5	6	NG	NG		<i>U. crassus</i> *	T2	226000785340	65	28	39.5
5	6	NG	Dead		<i>U. crassus</i>	T3	226000529755	69	29	44.5
5	6	NG	NG	x	<i>U. crassus</i>	T4	226000733575	59	25	39.5
5	6	NG	NG	x	<i>U. crassus</i> *	T5	22600733529	67	29	42
5	6	G	G	x	<i>U. crassus</i> *	TF2	226000125809	52	24,5	34
5	6		NG	x	<i>U. crassus</i> *	T72	Not tagged	59	25	37
5	6		G	x	<i>U. crassus</i> *	T73	Not tagged	67	43	29
5	6			NG	<i>U. crassus</i> *	T74	Not tagged	79	51	34
5	6			NG	<i>U. crassus</i> *	T75	Not tagged	71	31	44
5	6			NG	<i>U. crassus</i> *	T76	Not tagged	76	34	45
5	6			NG	<i>U. crassus</i> *	T77	Not tagged	75	34	43
5	6			G	<i>U. crassus</i> *	T78	Not tagged	65	29	40
5	6			NG	<i>U. crassus</i> *	T79	Not tagged	68	26	40



Figure 10. Fish (*Salmo trutta*) detected in the River Torpe Kanal during the mussel investigation in May 2019.

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