The last pearl mussel stream in Denmark: Mussel monitoring and habitat evaluation in the Varde river

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Abstract

The freshwater pearl mussel (*Margaritifera margaritifera*) is one of the most endangered freshwater species in the world, and its population has declined drastically in recent decades. In Denmark, the situation of the species is unclear, as the last living specimen was found in the Varde river system in 1995.

This study investigated the current status and the potential for reintroduction of the freshwater pearl mussel in the Varde system by: (i) dive monitoring to search for live mussels, and (ii) assessment of habitat quality at several sites in the main river Varde and its tributaries.

The diving did not yield any live mussels but confirmed the former presence of the species in the system by discovery of empty shells and shell fragments. The habitat assessment showed that most sites in the Varde River downstream of Karlsgårde reservoir are unsuitable for the development of juvenile mussels due to high levels of mobile sand and partly low oxygen levels in the interstitial. Conversely, high flow velocities and the lack of medium grain sizes made most of the sites in Ansager Å and Grindsted Å unsuitable. The oxygen conditions in the interstitial of the upper part of Holme Å seemed insufficient for the survival of juvenile mussels, but improved within the restored stretch further downstream. Suitable substrate conditions were additionally found in the Varde upstream of Karlsgårde reservoir and in the Linding Å. The availability of host fish has improved in recent years due to the removal of migration barriers in the Varde system.

The results indicate that the planned restoration measures in the Varde system reconnecting seven meanders are unlikely to cause any damage to the mussel population. However, supportive measures such as translocation, release of artificially infested host fish or breeding programs would be required to restore a viable population. Selection of a suitable source population with high genetic similarity to the original Varde population is essential. Prior to any reintroduction, conditions at target sites should be assessed using bioindication systems with juvenile mussels.

Further restoration efforts should focus both on improving habitat quality, such as reducing sediment input and restoring natural sediment dynamics, a supplementation of finer and medium size gravels and on promoting a healthy population of host fish, such as Atlantic salmon.

1. Introduction

The freshwater pearl mussel (*Margaritifera margaritifera* L., FPM) is one of the most threatened freshwater species worldwide (Geist 2010; Lopes-Lima et al. 2017; Machordom et al. 2003; Young et al. 2001). Its distribution ranges from western Russia through Europe to North America with a focus on rivers and streams low in lime and nutrients, making it an ideal target species for conservation (Geist 2010). Over the last few decades, a sharp decline in both the number of individuals and populations has been documented, bringing this mussel species to the brink of extinction (Geist 2010; Lopes-Lima et al. 2017). Many of the remaining populations in Central and Southern Europe are small, highly fragmented, have low genetic diversity and lack recruitment (Geist 2010; Lois et al. 2014; Stoeckle et al. 2017) as a result of numerous pressures such as habitat alteration, pollution, intensive land use, exploitation and declining host fish populations (Degerman et al. 2013; Moorkens 2010; Skinner et al. 2003). Most of the Central European populations of FPM had already declined by 90 % by 1990 (Bauer, 1988), mostly due to unsuccessful recruitment of juveniles. The highly complex life cycle of the species includes an obligatory parasitic phase of its glochidia larvae on a host fish for several months where it metamorphoses into a juvenile mussel (Taeubert and Geist 2017; Young and Williams 1984). Suitable host fish are exclusively salmonids (Geist et al. 2006; Österling and Larsen 2013; Taeubert et al. 2010; Young 2006), which can differ between populations: some show an specialization on brown trout (*Salmo trutta*), some on Atlantic salmon (*Salmo salar*) and some populations are able to use both salmonid species (Geist et al. 2018; Karlsson et al. 2013; Salonen et al. 2017). After the juvenile mussels drop off the host, they need to develop in the interstitial zone for 5 – 7 years and therefore depend on a well-oxygenized stream bed through the infiltration of surface water. The juvenile phase is recognized as the bottle neck for most of the European populations since a sufficient oxygen supply to the interstitial zone over the whole juvenile period is often hindered by high amounts of fine sediment clogging the inter-gravel pore spaces (Denic and Geist 2015; Geist 2010; Geist and Auerswald 2007; Österling et al. 2008). As a highly specialized and sensitive species, *M. margaritifera* has strict habitat requirements, which are often not (completely) fulfilled. Particularly, the micro-habitat within the stream substrate needs to be both stable enough to prevent detachment of specimens during high floods but at the same time inter-gravel pores need to contain low amounts of fine sediments to ensure oxygen supply into the interstitial (Denic et al. 2023; Geist and Auerswald 2007; Hastie et al. 2000; Hauer 2015; Quinlan et al. 2014; Scheder et al. 2015). However, restoration efforts are often based on subject opinions rather than scientific evidence, as multiple standardized approaches for monitoring freshwater pearl mussel populations have been developed across Europe (Geist 2015; Geist and Hawkins 2016). To address this problem, a guidance standard on monitoring *M. margaritifera* populations and their environment has been established (Boon et al. 2019; British Standards Institution 2017).

The biggest remaining populations of FPM in Europe are found in remote areas of Northern Europe, particularly in Scotland, Ireland, Sweden and Finland (Cosgrove et al. 2016; Moorkens 2010; Oulasvirta et al. 2017; Söderberg et al. 2009), with the lowest degree of anthropogenic impacts on the FPM streams. Here, populations display a high genetic diversity (Geist and Kuehn 2008; Geist et al. 2018; Geist et al. 2009), while the remaining populations in Central Europe are highly structured and low in genetic diversity (Stoeckle et al. 2017; Zanatta et al. 2018).

The current situation of the species in Denmark remains unclear. The only stream with documented historic occurrence of the FPM is the Varde river system in Western Jutland (Andersen and Wiberg-Larsen 2017). However, the last living individual was found at the Varde Sommerland in 1995 (Deacon, pers. comm.). Since then, multiple surveys only detected empty shells and no more records of any living specimen. Andersen and Wiberg-Larsen (2017)found eDNA from FPM in the main channel of River Varde, specifically at Varde Sommerland and Vagtborg. Rasmussen et al. (2023) conducted another eDNA survey in several of the river Varde tributaries. These recent positive detections in the Varde main stream may indicate the presence of few living individuals, but it is also known that the shells of dead mussels can release DNA during their decomposition (Geist et al. 2008), which may also explain the observed weak signals. However, as shown by Rasmussen et al. (2021), old and highly decomposed shells do not shed enough DNA to be detected by the method used, so if the eDNA signals are from decomposing shells, these individuals must have died within more recent years. Consequently, determining whether they originated from living specimens remains uncertain until living individuals are found. The recent efforts to improve freshwater habitats in Denmark, e.g. by removing migration barriers to facilitate fish migration, or the restoration of salmonid spawning grounds might have mitigated some of the negative historic impacts and resulted in improvement of FPM habitat. An evaluation of potential suitable habitats for FPM juveniles is therefore necessary in order to determine the next steps for conservation and restoration of FPM in the Varde system. The uncertainty whether living specimen of the FPM are present in the Varde river poses a problem for the forthcoming restoration measures. When the original meanders are reconnected, any remaining individuals could become damaged.

The purpose of this study was therefore to i) survey stretches within the Varde main stream in search for remaining, living FPM individuals, including the site of the last documented living specimen; ii) to monitor habitat conditions crucial to FPM over a range of sites within the Varde main stream and its tributaries to identify potential suitable sites for FPM juveniles, iii) providing a knowledge transfer to local stakeholders by demonstrating the habitat survey methods in the field and through an online workshop in October 2024 (see also Appendix).

2. Material and methods

2.1. Project area and sampling sites

A total of 23 sampling sites within the Varde river catchment were sampled in August 2024 by a team from the Aquatic Systems Biology Unit of Technical University of Munich, Germany (J. Geist, R. Hoess, A. Dobler, M. Tille, supported by the student assistants U. Shah and P. Schwarzenbeck). The sampling team was accompanied in the field by M. Deacon (all sites), F. Sorensen and J. Rasmussen (some of the sites). Of the sampling locations, 11 sites were located in the Varde main stream, of which three sites were located upstream of the Karlsgårde reservoir (Varde_us) and eight sites downstream of the Karlsgårde reservoir (Varde_ds). Seven stretches within Varde_ds will be filled up in the course of a reconnection of seven meanders to the Varde, that were disconnected from the stream channel in 1929. Those seven stretches and one additional stretch located at "Varde Sommerland", where the last documented living FPM was found, were intensively searched for mussels. Another 11 sites in four tributaries of the Varde were selected for the habitat assessment, including three sites in the Ansager Å, two sites in the Grindsted Å, six sites in the Holme Å and one site in the Linding Å (Figure 1). The land use intensity within the area varied between and along streams, with a high degree of agricultural land use and pastures as well as some remote stream stretches surrounded by marshland and forest or heathland. During the field survey, cattle grazing on the stream banks with a direct access the stream, causing point source soil erosion of trampled areas was observed mainly in the Varde_ds. Another direct source of fine sediments to the Varde main streams were polluted side channels delivering high loads of ochre and suspended particles. High ochre concentrations were obvious at the majority of sites. Fish farms adjacent to the stream are impacting the upper of Holme Å. Concerning stream morphology, particularly below the Karlsgårde reservoir the Varde is channelized, resulting in high current velocities and stream incision, which was also observed in stretches of the Ansager. Bank erosion seems to be another important fine sediment source in such stretches. The substrate was variable between sites, it often consisted of a high content of (mobile) sand between larger stones (e.g. Varde_ds), while sites that were located at rapids restored as salmon spawning ground had a high gravel content. The whole area has been subject to major river restoration effects to improve fish passage for migratory fish species such as sea trout (*Salmo trutta trutta*) and Atlantic salmon. In particularly, Holme Å was reconnected to its former channel by 2021 after its water had been diverted into the Karlsgårde reservoir for decades for hydroelectric power generation. For comparison with intact FPM habitats with recent recruitment, we used data from the Lutter stream in Northern Germany, sampled in August 2022 and data from a range of European FPM population of various population status (see Geist and Auerswald 2007).

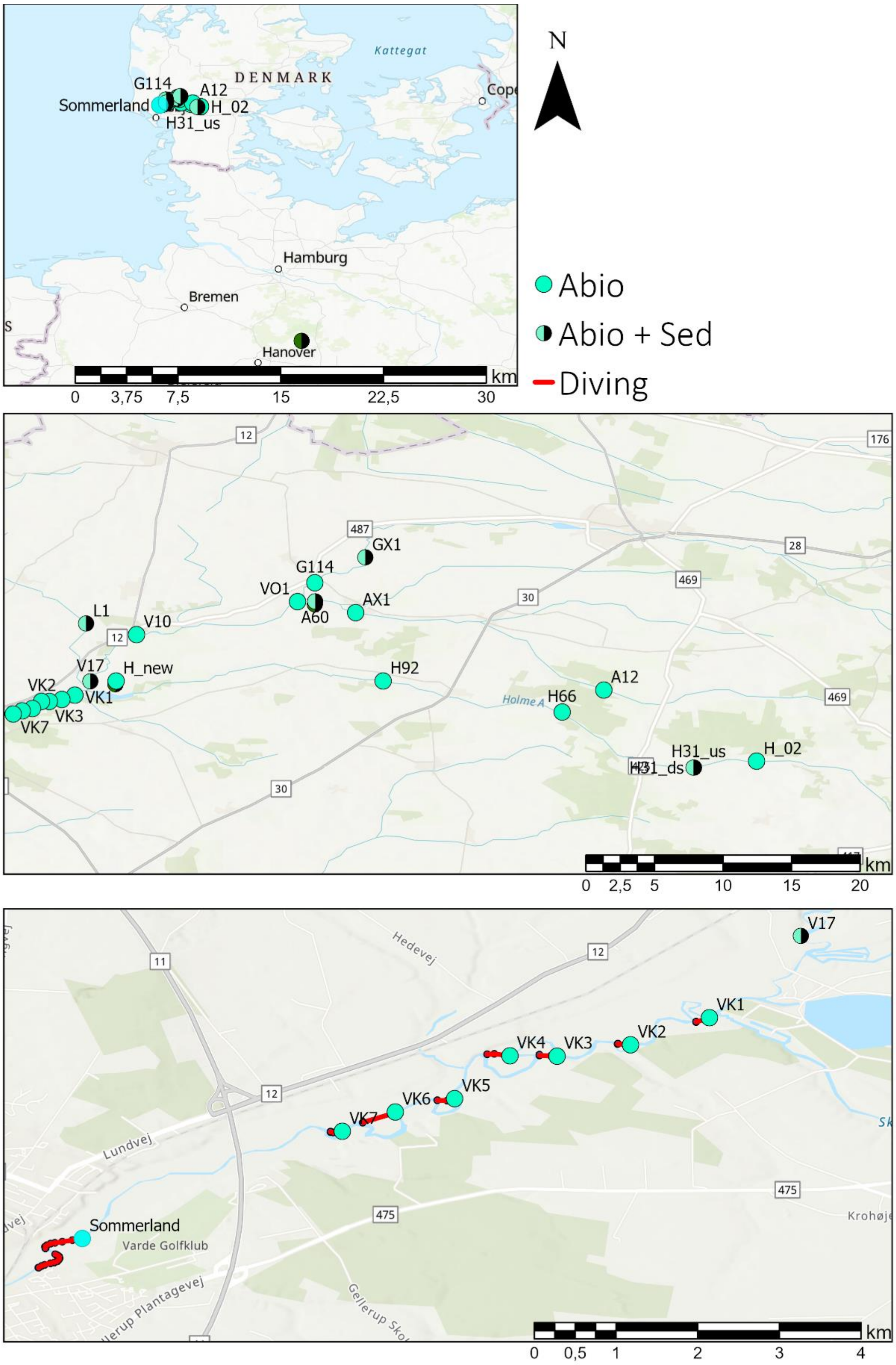

Figure 1: Map of the sampling sites in the Varde river system in Western Denmark and the Lutter stream in Northern Germany; fully colored circles indicate sites were only physico-chemical habitat assessment was conducted, half colored circles indicate sites were both physico-chemical habitat conditions and substrate composition were assessed; red lines indicate diving stretches searched for FPM.

2.2. Diving survey

Mussel monitoring was carried out in eight stretches of the Varde main stream (Varde_ds). Due to the expected water depth of >1m, strong current and high turbidity, a conservative monitoring approach by wading was not feasible, so the monitoring was performed by scuba diving in accordance with the existing national German scientific diving regulations (DGUV-Regel 101-023 "Einsatz von Forschungstauchern"). Focus of the search was set on both sides of the river as those areas consisted of the most suitable sediment for freshwater pearl mussels. Due to the strong current, the diver was attached to a guiding line, which was connected by a carabiner to a safety line stretched across the river, allowing the diver to move both across and with the current (see Figure 2).

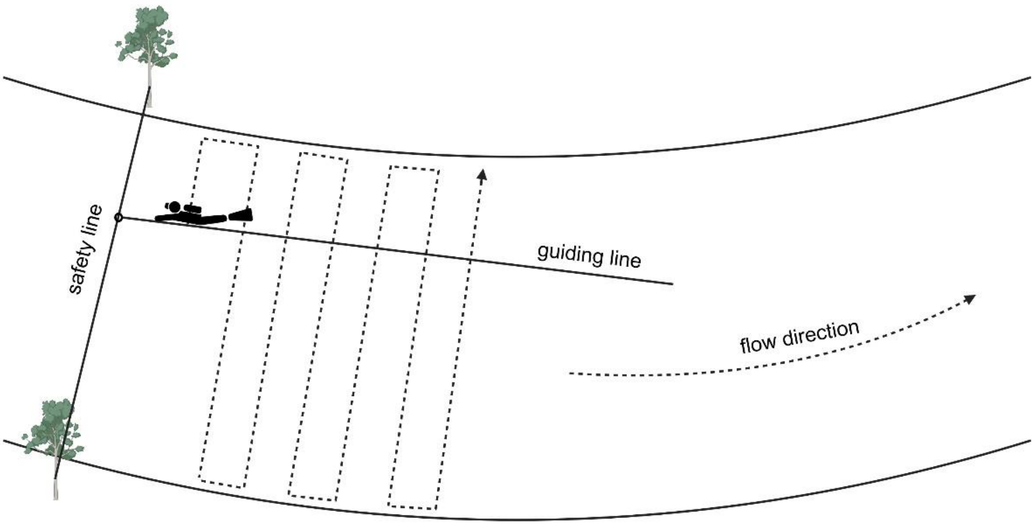

Figure 2: Illustration of the systematic dive monitoring approach. Solid lines show the safety line system, which is stretched across the river, and the guiding line, which allows the diver to move both across and with the flow direction (dashed line).

2.3. Habitat assessment

To evaluate the suitability of the habitat for *M. margaritifera*, a focus was placed on stream bed and interstitial habitat quality since this habitat was previously found to be a key bottleneck in the life cycle of the species elsewhere (Geist and Auerswald 2007). The habitat assessment followed the CEN standard DIN EN 16859 "Water quality - Guidance standard on monitoring freshwater pearl mussel (*Margaritifera margaritifera*) populations and their environment" (Boon et al. 2019; British Standards Institution 2017). At each sampling point, the oxygen concentration (O_2, in mg/L), temperature (T in °C), conductivity (cond. in µS/cm, corrected to 20°C) and pH value were measured using a Multi-3430 G (WTW, Weilheim, Germany) three times in the open water (FW) and from three water samples taken from a depth of 5 and 10 cm in the substrate (INT) at each sampling site. Following Pander et al. (2015), the interstitial samples were taken at three points along the watercourse cross-section using a pointed metal tube with perforated opening at the end and an attached silicon tube. The metal tube is inserted into the stream bed to a certain depth and a standardized volume extracted from the interstitial pore space using a syringe. The redox potential (Eh, in mV) was measured according to Geist and Auerswald (2007) using a pH 3110 meter (WTW, Weilheim, Germany) with a platinum electrode against an $Ag/AgCl_2$ reference electrode three times in open water and at three locations *in-situ* at a substrate depth of 10 cm. A pocket penetrometer (Ejikelkamp, Agrisearch Equipment, Giesbeek, Netherlands) with custom-made adapter discs was used to measure the penetration resistance at 18 points per sample site to analyze the surface sediment consolidation (= penetration resistance, Pen in kg/cm^2) according to Geist and Auerswald (2007).

To characterize the hydromorphological parameters of the sampling site, the flow velocity was measured at three points along the watercourse cross-section using a Flowtherm NT flow meter with a vane wheel (Höntzsch, Waiblingen, Germany) at the water surface (vo, m/s) and 2 cm above the substrate (vu, m/s). The corresponding water depths were measured to an accuracy of 0.5 cm using a measuring rod. In addition, the wetted width was recorded at the sample points. The turbidity (TURB, in NTU) was determined using a Turb 430 IR/SET measuring device (WTW, Weilheim, Germany).

At six sites (H31_us, H31_ds, GX1, A60, L1, V17), three substrate samples were taken along the river transection using a gravel sledge as presented in Pander et al. (2015). This sampler yields a mixed sample from the upper 10 cm of the stream substrate. Substrate samples were separated into the following grain size fractions by wet sieving using a AS 200 digit sieving machine (Retsch GmbH, Haan, Germany): > 20 mm, 6.3 - 20 mm, 2.0 - 6.3 mm, 0.85 - 2.0 mm and < 0.85 mm. The fraction < 0.85 mm is defined as fine sediment throughout the text. The individual fractions were dried at 102 °C and then weighed to the nearest 0.5 g.

2.4. Data analysis

To assess the connectivity between surface water and interstitial water as a key characteristics of functional juvenile habitats for FPM, parameter values measured in the interstitial were subtracted from the FW values, following the approach of Geist and Auerswald (2007). The obtained delta values (Δ) were included into the abiotic data set.

Substrate composition was assessed by comparing the weight percentage of fractions in the total weight of the sample. The geometric mean diameter (dg, in mm) of the sample was assessed following Sinowski and Auerswald (1999), using the following formulas:

$$dg = \exp(a)$$

$$a = \sum_{i=1}^{n} f_i * \ln(M_i)$$

with dg = geometric mean diameter, n = number of particle size fraction, f_i = mass fraction of the i-th fraction and M_i = modal size of the i-th fraction. To visualize these data, cumulative sieve lines for each sampling site were generated by cumulative addition of the proportion of a certain grain fraction in a sample.

Abiotic habitat parameters were analyzed with the open source software R (Version 4.1.0, www.r-project.org) using the user interface RStudio (Version 1.4.1717) and in PRIMER v7 (Plymouth Marine Laboratory, Plymouth, UK). Univariate parameters were tested for significant differences between streams using ANOVA with post-hoc Tukey-test if data were normally distributed (tested using Shapiro-Wilk test) and variances homogeneous (tested using Levene-test). If these requirements were not met, Kruskal-Wallis-test with post-hoc pairwise Mann-Whitney U-test were computed. A multivariate dataset comprising all physico-chemical parameters, delta values, surface flow velocity and turbidity was normalized by subtraction the mean and dividing by the standard deviation. The normalized data set was analyzed using Principal Component Analysis (PCA) and Analysis of Similarity (ANOSIM) to test for significant differences between streams based on Euclidean Distance. A significance level of $\alpha = 0.05$ was applied for all statistical analysis.

3. Results

3.1. Diving survey

Eight stretches with a total of 25.459 m^2 were monitored by scuba diving within 19.0 hours of diving time. No living mussels were observed, but five empty *M. margaritifera* shells and some fragments (Figure 3A) were found in the stretch "Sommerland" where the last living mussels were found in the year 1995 (M. Deacon, pers. comm.). In the other stretches, only empty shells of other freshwater mussel species such as *U. pictorum* and *A. anatina* were found. The Varde system is also known to host living populations of those two species, but these mostly occur in other habitats than FPM and were therefore not recorded within the area surveyed by diving.

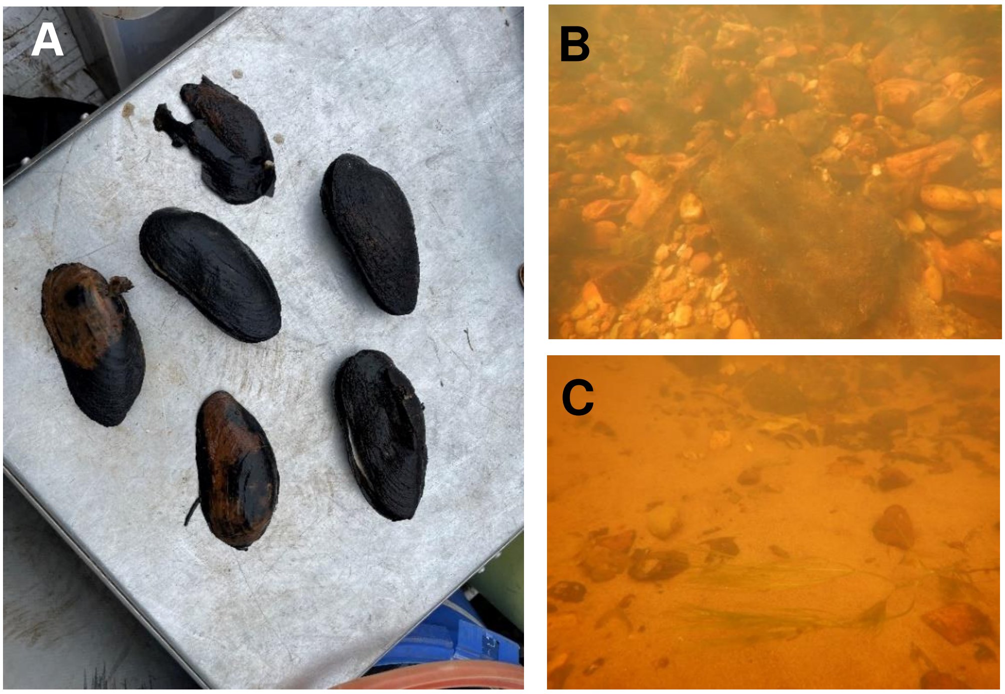

Figure 3: A) Photo of FPM shells (fragments) found during the diving survey within the Sommerland stretch; B) and C) Photos of the variation in surface stream bed composition within the Danish study streams.

3.2. Habitat assessment

Mean values of all parameter values measured within the study stream are given in Table 1. Abiotic habitat conditions differed significantly between all study streams except for Holme and Grindsted (ANOSIM, R = -0.15, p > 0,05), Grindsted and Varde_ds (ANOSIM, R = -0.01, p > 0,05) and Linding and Varde_ds (ANOSIM, R = 0.18, p > 0,05). Large, significant differences (ANOSM, R 0.51 – 0.99) were found between the Danish streams and the Lutter, which was mostly driven by differences in water temperature and oxygen concentrations in the open water (Figure 4). Water temperature in the Lutter, sampled in August 2022 was on average 4 °C warmer than in the Varde system in August 2024, leading to a slightly lower O_2 concentration in the Lutter (Table 1, Figure 4). However, O_2 concentration > 8,00 mg/L and redox potential > 470 mV indicated oxic conditions in the surface water of all study streams. Independent of the temperature differences, abiotic conditions in most of the tributaries of Varde and in the main stream upstream of Karlsgårde reservoir showed similarities with the Lutter river (Figure 4).

Table 1: mean ± sd of abiotic parameter values measured in the open water (FW) or in 5 or 10 cm substratum depth.

	Lutter	Varde_us	Varde_ds	Holme Å	Ansager Å	Grindsted Å	Linding Å
N (sampling sites)	5	3	8	6	3	2	1
O_2 FW [mg/L]	8.79 ± 0.16	9.68 ± 0.11	9.34 ± 0.12	9.52 ± 0.13	9.98 ± 0.15	9.62 ± 0.02	9.91 ± 0.00
O_2 5cm [mg/L]	3.47 ± 1.93	6.26 ± 2.63	2.08 ± 2.87	4.50 ± 2.47	7.95 ± 2.60	4.66 ± 1.70	9.54 ± 0.26
O_2 10 cm [mg/L]	NA	5.48 ± 3.20	NA	3.01 ± 2.11	7.33 ± 2.32	4.18 ± 2.51	8.00 ± 2.26
ΔO_2 5 cm [mg/L]	5.32 ± 1.90	3.42 ± 2.67	7.26 ± 2.89	5.02 ± 2.46	2.03 ± 2.49	4.96 ± 1.69	0.37 ± 0.26
ΔO_2 10 cm [mg/L]	NA	4.21 ± 3.20	NA	6.50 ± 2.09	2.65 ± 2.23	5.44 ± 2.51	1.91 ± 2.26
T FW [°C]	17.8 ± 0.6	14.2 ± 1.3	14.3 ± 0.6	13.2 ± 1.4	11.3 ± 1.1	14.6 ± 0.4	15.6 ± 0.0
T 5 cm [°C]	19.1 ± 0.4	16.4 ± 0.8	17.6 ± 2.3	15.4 ± 1.7	13.8 ± 1.1	16.1 ± 0.5	17.0 ± 0.2
T 10 cm [°C]	NA	16.4 ± 0.6	NA	15.3 ± 1.2	13.5 ± 1.6	16.2 ± 0.5	16.9 ± 0.8
ΔT 5 cm [°C]	-1.3 ± 0.4	-2.2 ± 0.9	-3.2 ± 2.2	-2.2 ± 1.2	-2.5 ± 0.6	-1.6 ± 0.3	-1.4 ± 0.2
ΔT 10 cm [°C]	NA	-2.2 ± 0.9	NA	-2.1 ± 0.9	-2.2 ± 0.8	-1.6 ± 0.4	-1.3 ± 0.8
cond. FW [µS/cm]	148 ± 1	234 ± 5	245 ± 3	220 ± 12	92 ± 98	238 ± 3	290 ± 0
cond. 5 cm [µS/cm]	175 ± 38	263 ± 72	439 ± 211	235 ± 32	183 ± 44	275 ± 44	296 ± 7
cond. 10 cm [µS/cm]	NA	291 ± 102	NA	242 ± 48	168 ± 34	265 ± 30	300 ± 20
Δcond. 5 cm [µS/cm]	-27 ± 38	-30 ± 74	-194 ± 212	-15 ± 34	-61 ± 121	-37 ± 43	-6 ± 7
Δcond. 10 cm [µS/cm]	NA	-57 ± 103	NA	-22 ± 46	-76 ± 122	-27 ± 29	-10 ± 20
pH FW	7.3 ± 0.1	7.0 ± 0.0	7.1 ± 0.0	7.1 ± 0.0	6.9 ± 0.1	7.0 ± 0.0	7.3 ± 0.0
pH 5 cm	7.0 ± 0.3	6.9 ± 0.1	7.0 ± 0.3	6.7 ± 0.3	6.5 ± 0.4	6.7 ± 0.3	7.2 ± 0.1
pH 10 cm	NA	6.8 ± 0.1	NA	6.8 ± 0.7	6.4 ± 0.6	6.6 ± 0.2	7.1 ± 0.4
ΔpH 5 cm	0.3 ± 0.3	0.0 ± 0.1	0.1 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.3 ± 0.3	0.1 ± 0.1
ΔpH 10 cm	NA	0.1 ± 0.1	NA	0.3 ± 0.7	0.5 ± 0.5	0.3 ± 0.2	0.2 ± 0.4
Eh FW [mV]	545.7 ± 24.4	512.7 ± 24.3	472.8 ± 53.7	490.5 ± 19.3	520.7 ± 77.2	509.5 ± 1.6	471.0 ± 0.0
Eh 5 cm [mV]	440.6 ± 82.3	441.6 ± 78.5	322.8 ± 143.4	315.9 ± 85.9	420.3 ± 146.8	372.7 ± 24.1	344.7 ± 28.3
Eh 10 cm [mV]	NA	398.7 ± 85.2	NA	254.2 ± 96.4	438.6 ± 144.7	333.8 ± 9.7	275.7 ± 14.8
ΔEh 5 cm [mV]	105.1 ± 78.9	71.1 ± 71.1	150.0 ± 148.9	174.6 ± 80.8	100.3 ± 76.6	136.8 ± 25.4	126.3 ± 28.3
ΔEh 10 cm [mV]	NA	114.0 ± 83.8	NA	236.3 ± 91.6	82.1 ± 113.7	175.7 ± 9.6	195.3 ± 14.8
Vo [m/s]	0.25 ± 0.27	1.22 ± 0.42	0.38 ± 0.13	0.73 ± 0.34	0.80 ± 0.32	0.85 ± 0.22	0.38 ± 0.12
TURB [NTU]	6.42 ± 0.92	11.99 ± 0.98	21.55 ± 3.72	8.33 ± 5.32	11.60 ± 4.65	9.10 ± 1.82	14.21 ± 0.37
Pen [kg/cm²]	0.51 ± 0.27	1.14 ± 0.58	0.32 ± 0.27	1.28 ± 0.59	1.00 ± 0.48	0.87 ± 0.73	0.50 ± 0.21

Regarding the Danish streams, differences could largely be attributed to a gradient in oxygen conditions in the interstitial. Highest O_2 concentrations of > 6 mg/L and > 5 mg/L in 5 and 10 cm substratum depth, respectively, could be measured in Ansager Å, Linding Å and Varde Å upstream of the Karlsgårde reservoir. Sites in the upper Holme and the Varde main stream downstream of Karlsgårde had lower oxygen levels already in 5 cm substratum depth and larger differences between oxygen concentrations in the open water and the interstitial, which is indicative of poor habitat quality for juvenile FPM.

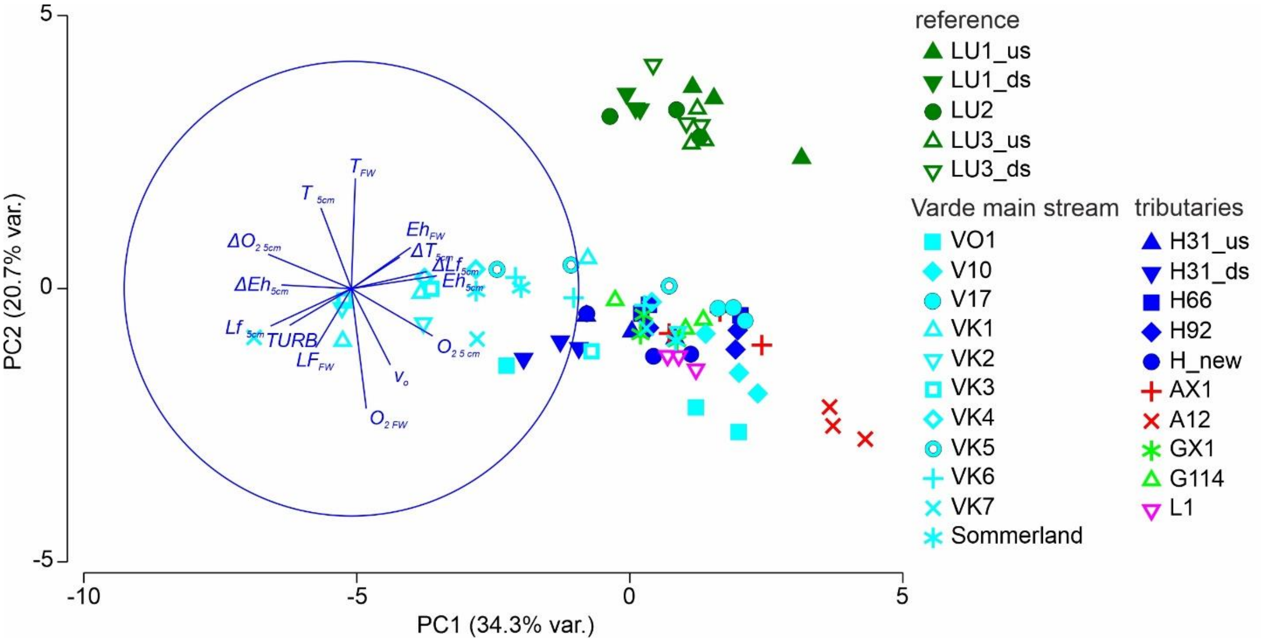

Figure 4: Principal Component Analysis (PCA) based on Euclidean distances performed on normalized values of abiotic parameters measured at the 23 sampling sites in the Varde system and five sites in the reference stream Lutter; different symbols represent different sampling sites in the reference stream (dark green), Varde main stream (turquoise), and the tributaries Holme Å (dark blue), Ansager Å (red), Grindsted Å (light green) and Linding Å (pink); small distances between symbols indicate small differences in the abiotic parameters between sampling sites; direction of the vectors indicate gradients of parameter values within the sampling sites while vector length represents the contribution of variable to the two principal components (PC1 and PC2), with the circle representing 100%.

The lower oxygen availability in the interstitial became also evident when comparing redox values (Figure 5). In all Danish streams except the Ansager, redox values in 5 and 10 cm substratum depth were significantly lower than in the open water (pairwise Mann-Whitney U-Test, Ansager: FW-5cm: $p > 0.05$; FW-10cm: $p > 0.05$; all other streams: p-values < 0.05,). In the Lutter, the Grindsted and the Linding all redox measurements were above 300 mV in 5 cm substratum depth, suggesting long-term oxic conditions. In the Ansager and Varde_us, only site A60 and V01 showed measurements around and/or below this threshold. In Holme, redox values showed a great variability between sites with site H66, H92 and H_new indicating oxic substrates while redox values were below 300 mV already in 5 cm substratum depth particularly at the upstream sites H31_us, H31_ds and H_02. A high variability in redox-condition in 5 cm substratum depth could also be observed for the Varde downstream of Karlsgårde. Here, variability was higher within sites, with low redox values below 300 mV close to the banks and high redox values > 300 mV in the middle section of the stream transect at the highest current velocity. Considering gradients in the redox potential within the substrate, all of the tributaries showed a steep decrease from the open water to 5 cm substratum depth and a further decrease to 10 cm substratum depth, which is similar to the patterns observed for non-function FPM-streams throughout Europe by Geist and Auerswald (2007). Steepest gradient could be observed in the upstream sites of Holme Å, in Grindsted Å and in Linding Å, although substrates in Grindsted Å seemed oxygenized also in 10 cm substratum depth. In contrast, the redox gradients in Ansager Å, in particular site A12, resembled more the gradients in potential-functional populations in Geist and Auerswald (2007). Redox potential in both 5 and 10 cm substratum depth seemed sufficient in most sites in the Varde_us and in the lower part of Holme, although they also showed declines in redox potentials from the open water into the interstitial, which are usually not observed in functional FPM-streams (Geist and Auerswald 2007).

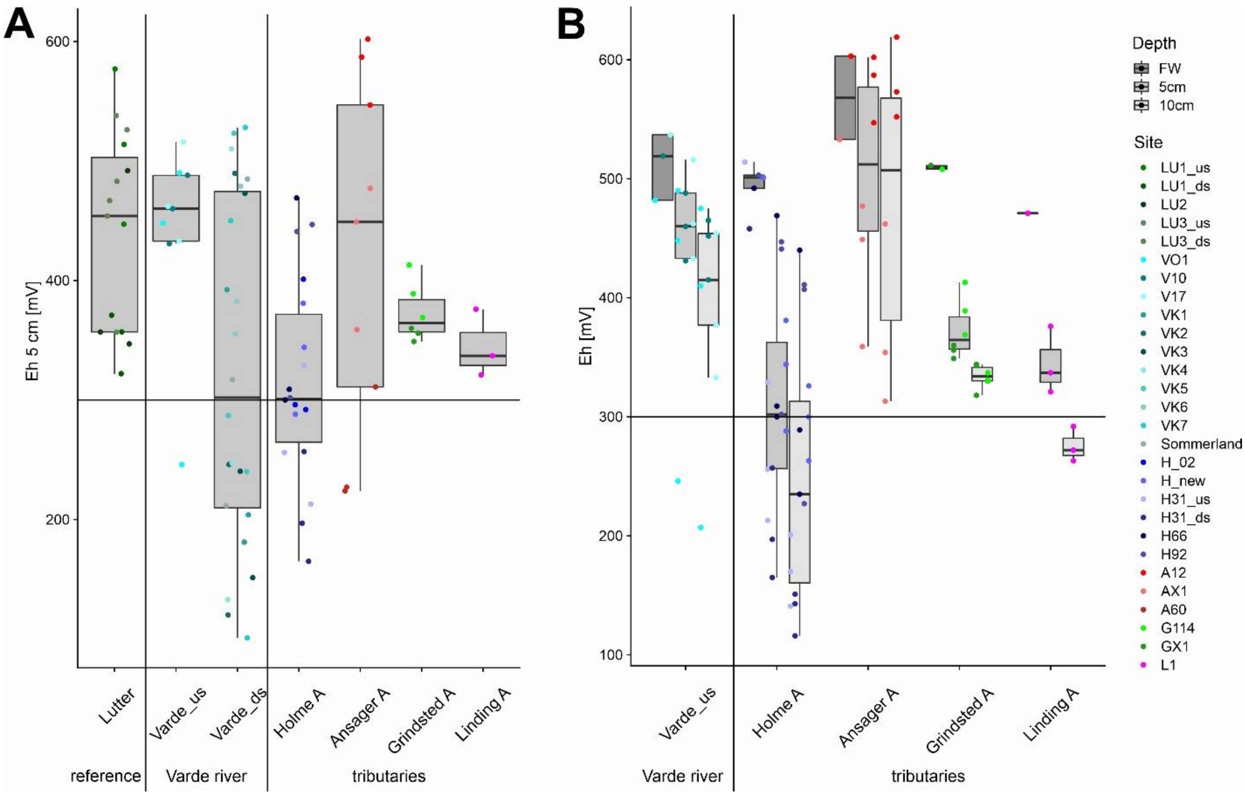

Figure 5: Box-Whisker plots of A) the redox potential measured at 5 cm substratum depth in the different study streams, including the reference stream Lutter and the Varde main stream below the Karlsgårde reservoir; and B) the redox potential measured in the open water (dark grey boxes), at 5 cm substratum depth (medium grey boxes) and at 10 cm substratum depth (light grey boxes) in the Varde main stream upstream of the Karlsgårde reservoir and in the tributaries; ,colored dots represent measurements from different sampling sites; the horizontal line represents the 300 mV threshold representing oxic (> 300 mV) or anoxic (< 300 mV) substrate condition.

Similar patterns could be observed for difference in electric conductivity in the surface water and the interstitial at 5 cm substratum depth (Figure 6). Small differences (< 20 µS/cm) between the two stream compartments, resembling the conditions in functional or potentially-functional FPM streams (Geist and Auerswald, 2007), could be observed in a majority of measurements in the Lutter, the Varde_us, and the site in the Linding, as well as several sites in the Ansager, the Grindsted and the lower part of the Holme. Differences in some sites in the Ansager, the Grindsted and the upper Holme, as well as the vast majority of measurements in the Varde_ds resembled more the patterns occurring in streams with non-functional FPM-populations.

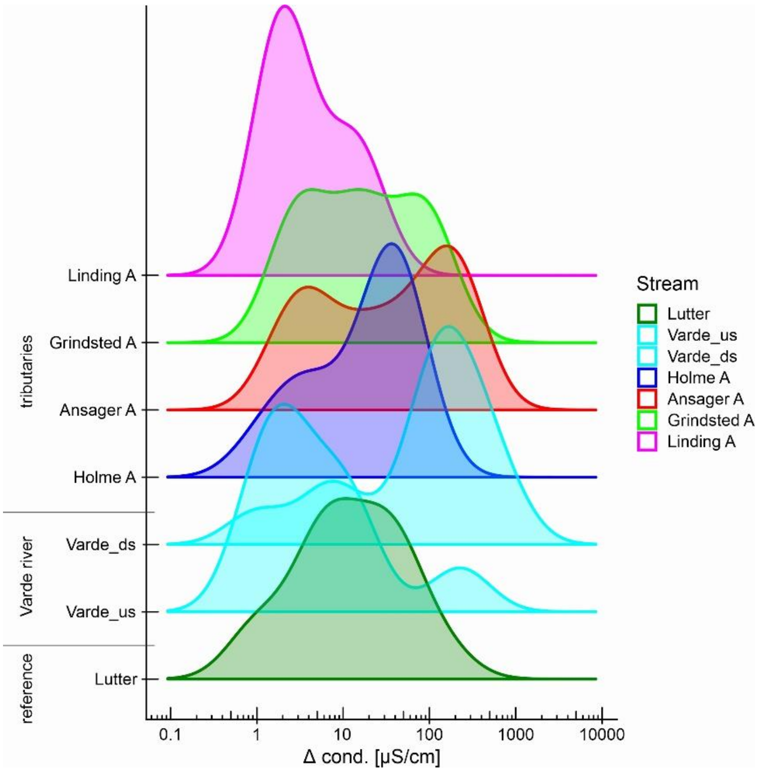

Figure 6: Density plots showing the distribution of the difference in electric conductivity (on a logarithmic scale) measured in the open water and in 5 cm substratum depth in the different study streams including the reference stream Lutter and the Varde main stream upstream of and below the Karlsgårde reservoir

Penetration resistance as a measure of stream bed compaction showed high variability within and between streams (Figure 7). In the Linding and the Grindsted it was similar as in the Lutter river (pairwise Mann-Whitney U-Test, p-values > 0.05), with mean values between 0.50 and 0.87 kg/cm² in the three streams. However, the Grindsted showed a high variability between the two sampling sites, with high values and a high variability at site GX1 and consistently very low values at G114. Highest mean penetration resistance of 1.28 kg/cm² was measured in the Holme, which was significantly high than in the Lutter, the Ansager, the Linding and the Varde_ds (pairwise Mann-Whitney U-Test, p-values < 0.05). Mean stream bed compaction in the Varde downstream of Karlsgårde was 0.32 kg/cm² and significantly lower than all other study streams (pairwise Mann-Whitney U-Test, p-values < 0.05). Highest penetration resistance in the Ansager was measured at site AX1 and several measurements at A60. Penetration resistance at site V17 was lower than in the more upstream sites of the Varde and more similar to the conditions within the river stretch below Karlsgårde reservoir.

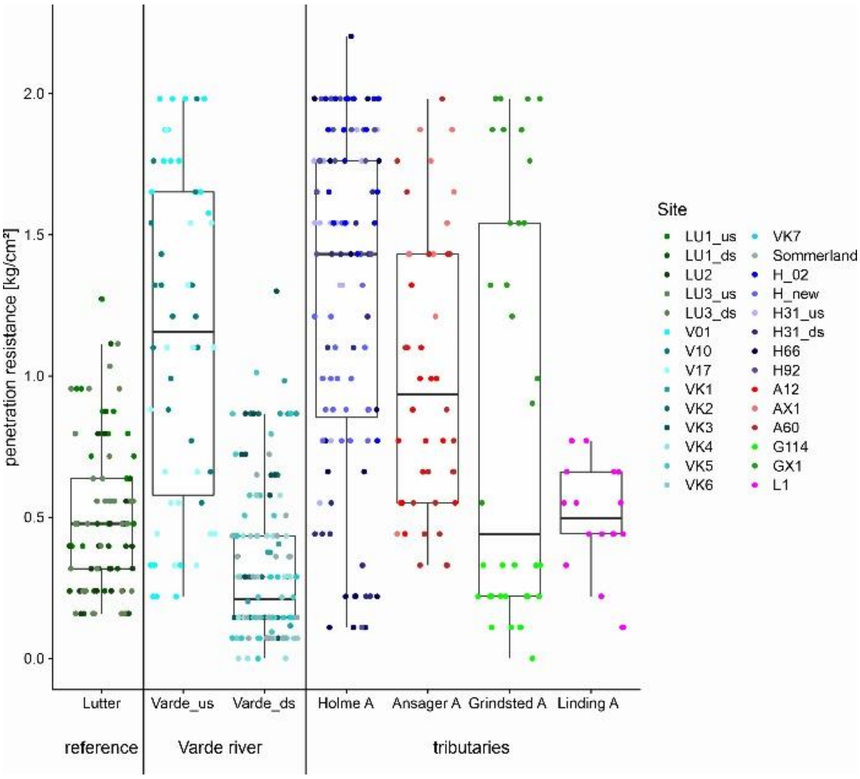

Figure 7: Box-Whisker plots of the penetration resistance of the substratum measured in the different study streams, including the reference stream Lutter and the Varde main stream below the Karlsgårde reservoir. Colored dots represent measurements from different sampling sites

Substrate composition of the substrate samples taken at five sites in the Lutter, two site in the Holme and one site in the Ansager, Grindsted, Linding and Varde_us are given in Table 2.

Table 2: mean ± sd of the proportion of particles of different grain size fractions and the mean particle diameter (dg) of substrate samples from the six study streams.

	Lutter	Varde_us	Holme Å	Ansager Å	Grindsted Å	Linding Å
N (samples)	5	3	6	3	3	3
< 0.85 mm [%]	47.28 ± 32.43	37.07 ± 54.5	21.80 ± 16.92	2.13 ± 3.00	4.57 ± 5.19	12.53 ± 10.34
0.85 - 2.0 mm [%]	4.00 ± 3.68	0.50 ± 0.46	3.80 ± 2.96	0.73 ± 0.21	0.53 ± 0.25	1.83 ± 1.37
2.0 - 6.3 mm [%]	7.20 ± 7.10	1.23 ± 1.16	12.53 ± 9.13	1.40 ± 1.42	0.93 ± 0.58	2.73 ± 1.70
6.3 - 20 mm [%]	16.34 ± 10.27	5.13 ± 6.18	21.58 ± 14.85	7.50 ± 1.25	14.23 ± 6.47	12.70 ± 3.70
> 20 mm [%]	25.20 ± 28.01	56.10 ± 48.82	40.30 ± 32.94	88.23 ± 3.67	79.73 ± 8.58	70.20 ± 11.98
dg [mm]	4.95 ± 5.1	14.64 ± 12.67	8.64 ± 7.45	27.42 ± 4.63	22.90 ± 7.21	14.92 ± 9.99

The mean proportion of fine sediments (< 0.85 mm) in the Danish streams varied from 2% in Ansager Å to 37% in the Varde_us, but showed a high variability between samples. Medium grain sizes were largely missing in substrate samples from rapids sampled in the Ansager and the Grindsted, as well as the Varde_us (Figure 8). In the Ansager and the Grinsted, the samples consisted almost exclusively of substrate particles > 6.3 mm (> 90%), resulting in a mean dg of 27.42 mm and 22.90 mm, respectively. In the Varde_us, the grain size distribution was highly variable within the sampling site, consisting either almost 100% of fine sediment or particles > 6.3 mm, depending if the sample was taken from a part of the stream bed covered with sand dunes or not. The variation in substrate composition at the stream bed surface is shown in Figure 3B & C. Only Holme and Linding showed a grain size distribution that resembled more that of the Lutter river, with a larger variation in grain sizes. However, all of these three streams had relatively high proportions of fine sediments. The Lutter, however, displayed a high variation between the five sampling sites, with one sample consisting almost exclusively of fine sediments. The proportion of fine sediments was also relatively high in the other Lutter samples, most likely due to the prolonged low flow condition prior to the sampling in August 2022.

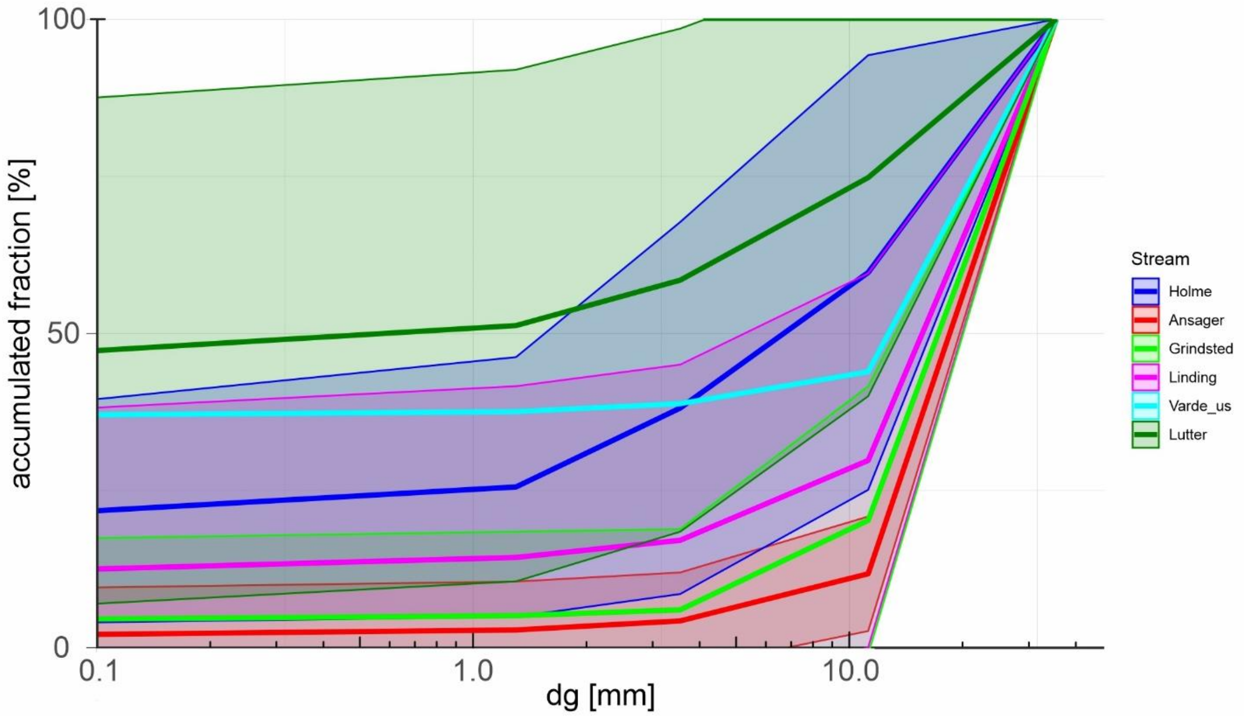

Figure 8: Cumulative textures of the mean grain size composition of sediment samples from the sites where substrate samples were taken; colored lines represent the mean over all samples per stream, colored, transparent areas represent the 95% confidence interval.

4. Discussion

The diving survey did not yield any living individuals, however, the recovery of several shell fragments clearly proved the previous occurrence of *M. margaritifera* in the Varde system. The shell fragments were already highly degraded and eroded, and therefore an age determination of the shells was not possible. This also indicated that the found individuals had been dead for some time, which questions the existence of any living individuals within the system. However, the positive results of the eDNA samples seem to indicate living individuals since a study by Rasmussen et al. (2021) showed that old and highly decomposed empty FPM shells do not yield enough DNA to generate false-positive eDNA results, even though leaching of DNA from decomposing shells appears possible (Geist et al. 2008). The diving survey was focused on seven stretches that will be affected by the channel restoration in the Varde, therefore not the entire channel length was searched. Consequently, whilst the findings of this study cannot completely exclude the occurrence of living specimens in the system, the findings clearly indicate that the planned restoration measures will likely not result in any damage to potentially occurring specimens in those stretches.

Even if there are few individuals remaining within the Varde system, they are apparently not able to successfully reproduce. This is most likely due to the strong anthropogenic impact on catchment land use and hydrology in the past. Considering all measured physico-chemical variables, the majority of sites within the Varde main stream downstream of the Karlsgårde reservoirs seem to be unsuitable for the development of FPM juveniles due to high amounts of mobile sand, destabilizing the stream bed habitat. This means that juveniles in these areas would be covered by sand dunes, having little refugia to prevent them from being constantly moved further downstream. Optimal habitat for freshwater mussels needs to provide flow refugia during high discharges, as shown by Allen and Vaughn (2010), Morales et al. (2006), (Sansom et al. 2020), and Strayer (1999) for several different mussel species, and for the FPM in particular by Denic et al. (2023), Hastie et al. (2000), Moorkens and Killeen (2014), and Scheder et al. (2015). This holds true for both juvenile and adult mussels. On the other hand, very high current velocities and resulting shear stress also make most of the sampled sites in Ansager Å and Grindsted Å unsuitable for the colonization by FPM. The impact of the strong currents can also be seen in the substrate composition: in both streams, the sampled rapids consisted mainly of very large grains > 20 mm. Although this led to a high oxygen supply to the interstitial and is likely beneficial for the use as salmonid spawning grounds, mussels are likely not able to steady themselves in the substrate as smaller grain sizes required for burrowing are missing. At the same time, the proportion of fine sediments also must not be too high to prevent stream bed clogging and limited oxygen supply to the interstitial. Geist and Auerswald (2007) propose a maximum content of particles < 1 mm of 20%, after analyzing habitat conditions in 26 FPM streams all over Europe. The penetration resistance gives a good indication of the state of stream bed colmation and substrate conditions, as it depends on grain size distribution but is also related to the population status in the study of Geist and Auerswald (2007): non-functional sites had a threefold higher penetration resistance than functional sites at an identical mean grain size diameter, indicating a pore space clogged with fine sediments. Clogging of interstitial pores decreases the oxygen availability in the interstitial as it decreases the supply of fresh oxygen that is consumed by (microbial) respiration processes (Malcolm et al. 2010). Respiration is further intensified by increased organic matter and nutrient concentration (Ingendahl et al. 2009). Several studies indicate that FPM prefer substrates with very low proportions of organic fine material (Österling et al. 2010; Tarr 2008). This is likely due to the higher biological oxygen demand during the break down of organic matter. Aquaculture facilities might be a significant point source for organic fine sediments (Fairchild and Velinsky 2006; Hoess and Geist 2021; Hoess and Geist 2022; Sindilariu et al. 2009). The impact of such a facility upstream of the sampling sites at the upper part of Holme Å were likely one of the reasons for the low values for the redox potential, despite the visual impression of the sites looked visually very promising, with diverse riparian vegetation and a gravelly stream bed. However, the steep gradients in redox potential indicated anoxic conditions already in 5 cm substratum depth. The strong differences in electric conductivity between open water and the interstitial at these sites also hint towards a strong mineralization of organic matter and thus respiration within the interstitial. Further downstream, the substrate conditions within the Holme increased, particularly in the lower part that had been recently restored in 2021. The reconnection of the former channel and gravel supplementation together with the removal of migration barriers for fish also improved the situation for the host fish of the FPM. The measurements at the respective rapids indicated suitable spawning ground conditions. The remodeling of the aquaculture facility in the upstream part of the Holme therefore offers a promising chance for habitat improvement.

The substrate conditions in the Varde upstream of Karlsgårde and in the Linding also seemed more suitable for a functional FPM population: both the penetration resistance and the mean grain size diameter fall into the range found in functional FPM populations, as well as the very small differences in electric conductivity and the redox potential between the surface water and the interstitial (Geist and Auerswald 2007). In the Varde_us however, the conditions were highly variable within the sampling sites, indicating large difference between more suitable and unsuitable microhabitats.

In summary, good substrate quality in the Varde system can be found mostly at sites with hydraulic conditions unsuitable for the long-term establishment of freshwater pearl mussels, while at sites with lower shear stress, high fine sediment content and/or mobile sand indicate unsuitable juvenile habitat.

The results from the reference stream Lutter, one of the few examples for a successful restoration of a viable FPM population in Central Europe, only partly match the requirements for functional FPM streams. One reason for that might be that the sampling was conducted during low flow conditions in August 2022, that can increase the settling of suspended particles, and with high water temperatures. These conditions represent the worst-case for cool-water adapted species like the FPM. Also, the sampled sites did also include locations that are not inhabited by the species. This represented the natural habitat heterogeneity of streams. Still, the majority of sites showed good substrate quality even under sub-optimal meteorological conditions. The life history strategy of the FPM, including the high reproductive potential through the production of millions of glochidia per female offers a great opportunity to colonized suitable habitats. The natural mortality within the first years is expected to be very high, as reported by Young and Williams (1984) and Buddensiek (1995) and also known from the multiple captive breeding programs (Geist et al. 2023). If juveniles happen to drop-off and develop at a suitable site, they will contribute to the reproductive potential and subsequently increase population numbers. At high population numbers, freshwater mussels help maintaining the habitat quality by removing suspended particles from the water column.

The availability of host fish is also crucial for a successful reproduction of FPM. However, host fish density was not found to be the main factor for the functionality of a FPM population, while fish community composition and the availability of a suitable host strains are more important (Geist et al. 2006). In the Varde system, many in-stream barriers were removed in the last decade, which highly increased natural fish migration, especially for the Atlantic salmon. Recent fish population surveys also show that juvenile salmon in particular are increasingly detected in the Varde system in the last years (Pedersen et al. 2022). This indicates a successful improvement of migration routes and, at the same time, the existence of suitable spawning sites with an exceptionally good oxygen supply to the interstitial, which is in line with the findings of this study. As both species rely on a well oxygenized stream bed, the results obtained using the presented methodology are relevant for both salmonids and mussels. Conservation measures should focus on both a good salmonid population and healthy FPM populations, as both taxa are mutual beneficial. While salmonids are obligatory for the reproduction success of FPM, *M. margaritifera* can support salmonids more indirectly. As filter feeders, FPM purify the water via biofiltration (Vaughn 2018; Zieritz et al. 2022). This reduction of nutrients could decrease habitat suitability for more generalist fish species, increasing competitiveness of more specialized species such as salmonids, as an opposite trend has been shown for some non-functional FPM streams (Geist et al. 2006). In addition, the burrowing behavior of FPM can improve spawning grounds, as bioturbation of sediments leads to an increase in sediment water and oxygen content (Boeker et al. 2016; Vaughn and Hakenkamp 2001), which is necessary for a successful recruitment of gravel-spawning fish such as salmonids.

Despite these promising developments and the suitable habitat conditions in some of the tributaries, the low substrate quality at the majority of sites with suitable hydraulic conditions still poses some challenges for a further improvement of the FPM habitat in the Varde system. These include high sediment inputs from catchment land use and aquaculture, changes in stream morphology and flow regime, like channelization and a low overall structural diversity, as well as high concentrations of ochre.

Successful and sustainable restoration of FPM habitats needs to consider both the channel and the catchment scale (Hauer 2015). This includes the introduction of larger boulders, as intensively done in Swedish steams cleared for timber floating (Frainer et al. 2017; Nilsson et al. 2014), or the flattening of stream banks to facilitate the deposition of fine material outside of the channel during high floods. Within the Lutter river, the construction of sedimentation basins before the outflow of heavily polluted drainage channels, as well as the use of a mill pond as sedimentation structure significantly reduced the amount of mobile sand within the stream, resulting in a re-establishment of FPM recruitment and increasing population size from about 1,800 to > 7,000 individuals within 12 years (Altmueller and Dettmer 2006). In south-eastern Bavaria, the restoration approach for the remaining FPM streams is comprised of a combination of all the above mentioned measures. A similar approach might also be feasible within the Varde system, e.g. by constructing sediment traps in problematic tributaries and flattening stream banks to reduce sand loads and establish self-dynamic desanding (Stelzer et al. 2023). Further extensivation of aquaculture and agricultural production in FPM catchments is needed to reduce fine sediment and nutrient inputs, e.g. from fish feed, over-grazing and trampling of stream banks by cattle and soil erosion (Hoess and Geist 2020). The planned re-connection of meanders in the channelized stretch between Karlsgårde and the city of Varde might also help to restore natural sediment dynamics, as this would increase flow heterogeneity, which is actually lacking in many parts of the Varde system. The ochre issue should be addressed by trying to modify the hydrological regime in order to raise groundwater levels in the floodplain to re-wet drained meadows (Sand-Jensen et al. 2006), which offers additional benefits such as increased water storage and the delivery of detritus food for the FPM (Brauns et al. 2021).

Recommendations for future steps

Since the last living individual of FPM in the Varde river was found 1995 and multiple surveys of the system including this one did not discover any surviving specimen, a re-establishment of a viable population would need support from some kind of augmentation measures. This could be the translocation of living individuals from a viable population, the release of artificially infested host fish, or a captive breeding program to rear juveniles to a certain age to bridge the critical phase in the interstitial. Similar measures are already taken all over Europe (Geist et al. 2023) and they show first positive effects in some populations (Dobler et al. 2024). The European FPM populations show a strong genetic structuring between and within drainage systems, which indicates a high degree of local adaption, including host fish use (Geist et al. 2018). A sustainable restocking program should try to use a source population of a high genetic similarity to the local Varde population. Since no living individual was found, specimens for museum records can offer a source for tissue and DNA of the original population that can be compared to genetic information of geographically close recent populations and select the one the closest related as a source population. Genetic analyses of old FPM samples from two specimens of the Varde system are currently ongoing at the Chair of Aquatic Systems Biology Unit of the Technical University of Munich.

Once a suitable source population is identified, the stream conditions at multiple target sites should be evaluated using translocated adult mussels or juvenile mussels exposed to the water column in net cages as proposed by Buddensiek (1995) or wooden boxes filled with sediment (Wagner et al. 2024) or in mesh tubes buried to the interstitial (Bílý et al. 2020). Such bioindication systems are widely used in Germany (Wagner et al. 2024) and the Czech Republic (Bílý et al. 2018; Černá et al. 2017). Such a long-term assessment using the target species can provide crucial information on the most suitable sites, in addition to the physico-chemical measurements carried out in this study which can help identify the most promising sites. Any restocking plans should always be combined with restoration efforts to improve habitat quality at target sites to further improve habitat conditions. Ongoing and planned restoration projects, such as those in the upper Holme, offer the opportunity to set suitable habitat conditions for the FPM as the main target. This would be a practical and effective approach to achieve measurable ecological and hydrological benefits.

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Appendix:

Figure 9: Impressions of the dive survey and the habitat assessment in the Varde system.