The first successful resettlement of *Margaritifera margaritifera* in Bavaria:

Evidence for the need of long-term monitoring data

Introduction

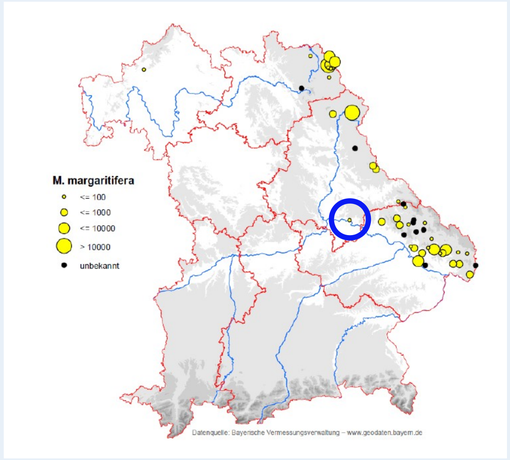
In Central Europe practically all populations of *Margaritifera margaritifera* are overaged. Starting in the 1980th, numerous attempts have been made to directly improve the age structure of pearl mussel populations (SIMON et al. 2015). Until now very few projects reported sustainable population increases or improvements in the age structure (ALTMÜLLER & DETTMER 2006, SIMON et al. 2015).

In Bavaria there are about 56 pearl mussel populations. In most mussel rivers, the last significant rejuventation occured more than 30 to 50 years ago. To the best of our knowledge, thousands of fish infected with glochidia and more than one million of captive-bred juveniles (mostly post-parasitic stage, but also captive bred subadults) have been released over the decades. In 2005 the Landschaftspflegeverband Regensburg started a project that became an exceptional success of reintroduction of *M. margaritifera* in a small former pearl mussel stream with very good environmental conditions.

The habitat quality was investigated prior to and accompanying the resettlement efforts, together with mussel surveys. Here we report long-term monitoring results to emphasize the role of an ongoing investigation program for a successful rejuvenation of mussel populations.

Study area

The studied stream is a small tributary of the Danube. The catchment area is NATURA2000 site. It is dominated by near-natural deciduous forests as well as spruce plantations. Part of the river valley is covered by extensively managed hay meadows. Besides *M. margaritifera,* protected species listed in Annex II of the EU Habitates Directive are yellow bellied toad (*Bombina variegata*), stone crayfish (*Austroptamobius torrentium*), large blue (*Phengaris teleius*) and dusky large blue (P. *nausithous*).



Recent populations of M. margaritifera in Bavaria and study area.

Monitoring and Mussel reintroduction

Initial and ongoing monitoring

In 2005, the historical and present abundance of *M. margaritifera* was documented from various reports and a thorough search. By then the population was practically extinct (BERGNER & SCHMIDT 2006).

In the same year, previous to the resettlement efforts, and again in 2010 and 2018, the habitat quality was investigated during low-flow periods in summer with the following measurements:

Water quality: pH, conductivity, temperature curves with data loggers (EL-USB 1), N and P parameters.

Hydromorphology: Water depths, channel width and flow velocity.

Substrate quality: Only optimal sites seemingly functional for juvenile mussels were examined.
Particle size distribution to a depth of 5 cm (subsequent washing and sieving);
Substrate compaction (hand-held penetrometer, Eijkelkamp);
Redox potential: Depth profile (platinum electrode against a reference Ag/AgCl2 electrode (GEIST & AUERSWALD 2007).
Oxygen availability: 4 iron nails of 16 cm length per site were driven into the substrate for 4 weeks. Rost formation indicated sustained availability of oxygen in the interstitial.

Mussel reintroduction

In 2005 one of the last adult mussels was found freshly dead. It was genotyped by the group of Jürgen Geist, TUM with the aim to find a new donor population for glochidia. Since 2006 each year indigenous fish were caught on-site, infested with glochidia from a regional donor-population with a similar genetic profile, and released immediately (until now a total of 1.000 fish). At the same time, post-parasitic juveniles, collected from infested fish on a fish farm, were released at sites with promising substrate (until now about 90.000 mussels).

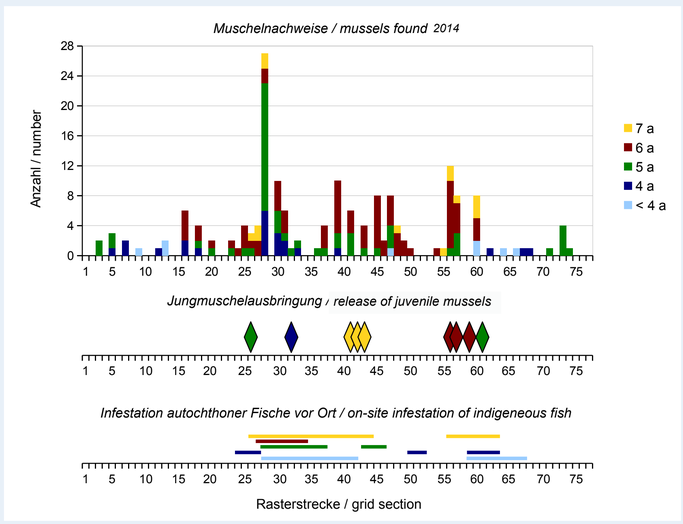
Population monitoring

After two 5 year old juveniles had been found in 2013 by chance, a grid search along the river was done in 2014 to assess the number, distribution and age structure of mussels. Using a viewing basket and torchlight, the first 5m of each 50m stretch was visually searched for 10 min, as far as possible without setting a foot in the river bed. The age of the juveniles was determined by counting of growth rings (HAUKIOJA & HAKALA 1978). In 2017 the survey was repeated using the identical sampling grid.

Results

Mussel population 2014

In the first survey 2014 at a stream length of 3.200 m 175 juveniles were found in 77 grid sections (0 to 5,4 mussels per m; mean: 0.45 per m). Population size was estimated to be **1.750 mussels** (parametric 95%-confidence interval: 1.057 to 2.443). The youngest mussel was 2 years old, the oldest 7 years, corresponding well with the beginning of repopulation measures. The distribution of age cohorts suggests that infestation of glochidia on resident fish as well as the release of juveniles worked: Juveniles in the upper part of the stream (grid sections 71-74, green columns) can only have been brought there by the resident fish, whereas in the middle part (grid sections 28-59) the occurrence of 4 and 6 year old juveniles might correspond with juvenile release sites.



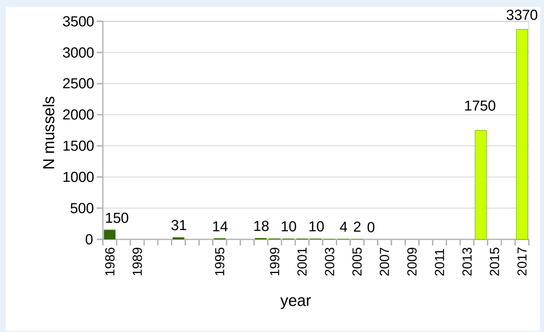
Distribution (number) and age (a) of juveniles per section grid in 2014 (upper diagram) and their probable provenience from releasing (middle diagram) and on-site infestation (lower diagram)

coloured rhombs - age of mussels released in the grid number in the corresponding year; coloured lines – age of mussels inserted in stretches in the corresponding year.

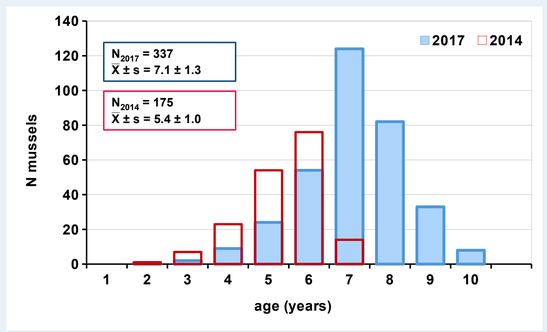
Mussel population 2017

In 2017 the recolonised stream range had expanded upstream. From 337 juveniles in 95 grid sections (0 to 8,8 mussels per m; mean 0.71 per m) a population size of **3.370 mussels** (95%-conf. int. 1.800 to 4.940) was estimated. Mussel ages ranged from 2 to 10 years. The mean age was 7.1 ± 1.3 in 2017 years compared to 5.4 ± 1.0 in 2014 (mean ± standard dev.). In both surveys the number of empty shells or dead mussels found suggests that annual mortality is below 1%.

In 2017, fewer mussels of the youngest age classes were found than in 2014. Either the searching conditions were less favourable in 2017, or fewer juveniles emerged in the later years.



Trend of the freshwater pearl mussel population
dark green columns - adults, bright green columns – juveniles.



Age structure of the mussel population 2014 (blank columns) and 2017 (filled columns).

N - number of mussels, x - mean and s - standarddeviation.

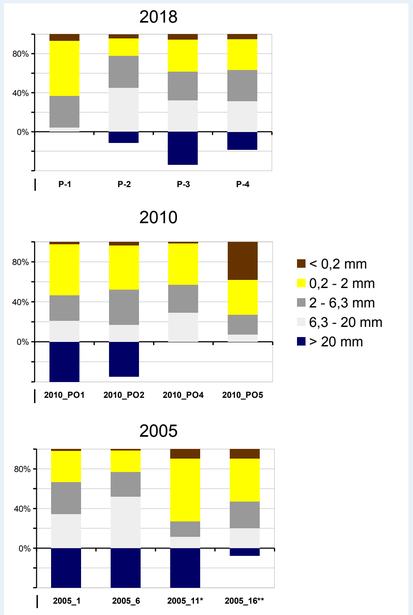
Trends of Habitat Quality in the substrate

The position of all sampling sites were recorded using GPS (Garmin 60CSx). However, river bed dynamics demanded to move some sites to adjacent spots over the years to constantly measure only favourable stream patches and substrates.

Fine sediment: In 2018, the unfavorable size class < 0,2 mm has increased in the substrates as compared to 2010 and 2005, but only in the downstream sites (lower site numbers). Seemingly there is a displacement of fine sediments from upstream sites to downstream sites. The larger size classes show no clear trends.

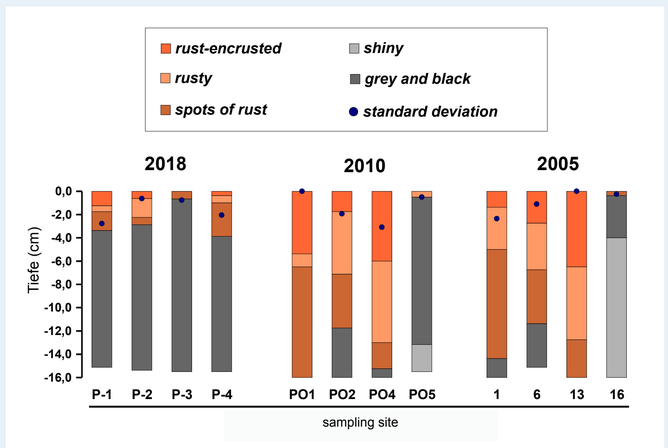
Oxygen availability: Rust formation on nails shows a greatly decreased oxygen availability in the present measurements in 2018. This again is true for the downstream sites only. Probably this relates to the fine sediment contents: Fine sediments in the substrate decrease water exchange and lead to oxygen depletion in the interstitial zone.

Redox potential: Generally the redox potential shows a decline in the substrate with increasing depth, as oxygen depletion gets more pronounced. The fall of redox potential from the free water to a depth of five cm is used as an indicator value: Less than 20 % decline indicates an elevated exchange of water within the substrate and a good habitat quality. In 2018 the decline was more than 20 % at all four sites, whereas in 2010 two out of four sites showed a lower decline and favorable conditions. The indicator is reasonably conservative: In 2018 the two measurements gave similar results. The standard deviation of the redox fall per date and site ranged from 8 to 35 %.



* sampling sites in the same stream section, but not identical to sites in 2018

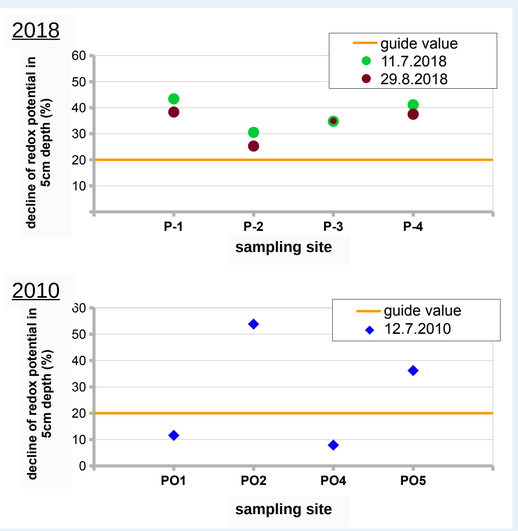
Percentages of particle size classes in relation to the sum of all classes < 20 mm. size > 20 mm: shown to a maximum of 40 % only.



Availability of oxygen in the substrat indicated by rust zons on iron nails in the years 2018, 2010 and 2005.

data: means from 4 nails per sampling site plus standard deviation (blue dots) of the sum of zones with rust.

exposition periods: 11.7. - 30.08.2018, 11.6. - 12.07.2010, 19.8. - 23.11.2005.



Decline (%) of redox potential in 5 cm depth compared to the free water body.

Dots and rhombs: Mean values from 3-9 measurements.
yellow line - reference value in functional pearl mussel streams (KILLEEN & MOORKENS 2013).

Conclusions

Despite the extraordinary successful establishment of juvenile mussels and the ongoing growth of the population, the monitoring data give rise to concern:

- The development of the age structure might indicate that the emergence of new juveniles is weakening.
- The substrate measurements might show an ongoing siltation, accompanied by decreasing oxygen supply, especially in the downstream sections of the stream.
- In total, an increased awareness of a possibly unfavorable development seems appropriate.

However, age structures of two years and substrate measurements at only four sites do only qualify for an initial suspicion.

The development of the population has to be monitored by regular surveys. Unexpected changes in population size and age composition could be a sign of deterioration of the habitat and necessitates cause determination.

The reduction of fine sediment deposition to natural levels (DENIC & GEIST 2014, MOORKENS 2010, ALTMUELLER & DETTMER 2006) and the conservation of suitable hydrodynamics and oligotrophic conditions are the keys to preserve or restore functional pearl mussel habitats.

Periodic monitoring of water and sediment quality is crucial for the successful management of species conservation projects. If habitat quality is deficient, the causes of impairment have to be found in the river and its catchment and appropriate mitigation measures have to be implemented.

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