

COMPARISON OF TWO DIFFERENT FIELD CAGES FOR SEMI-NATURAL REARING OF JUVENILE FRESHWATER PEARL MUSSELS, *MARGARITIFERA MARGARITIFERA* (LINNAEUS, 1758) (BIVALVIA: UNIONOIDEA: MARGARITIFERIDAE)

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ABSTRACT: Due to a lack of natural recruitment, the freshwater pearl mussel is on the brink of extinction in many parts of its distribution area. As a consequence, several breeding programs are in operation, many of them using semi-natural rearing methods. Especially during early life stages, several types of rearing devices are used to raise juveniles. However, there is no systematic comparison of different devices. In our experiment, we compared Buddensiek cages and sediment boxes. Each rearing device was filled with 30 one-year-old individuals. At 10 sampling sites three Buddensiek cages and three sediment boxes were exposed. Exposure started in June 2016 and ended in May 2017. Overall, survival of juveniles was higher in Buddensiek cages than in sediment boxes with 40–93% compared to 0–87%. Growth was comparable in both field cages. The study site specific variation indicates that suitability of field cages is determined by environmental conditions. Furthermore, handling effort differs between the tested field cages. The optimal operating conditions for each field cage are discussed.

KEY WORDS: freshwater mussel, *Margaritifera*, conservation, mussel propagation

INTRODUCTION

The freshwater pearl mussel (fpm) *Margaritifera margaritifera* (Linnaeus, 1758) is a highly sensitive freshwater bivalve, which is adapted to oligotrophic, cool primary rock streams with well sorted substrata and high water quality (HASTIE et al. 2000, GEIST & AUERSWALD 2007, ÖSTERLING et al. 2010, ÖSTERLING & HÖGBERG 2014, DENIC & GEIST 2015). Its particular habitat demands and complex life cycle, involving a parasitic stage on suitable host fish, make the species highly vulnerable to human disturbances (YOUNG & WILLIAMS 1984, COSGROVE et al. 2000). Consequently, the species has dramatically declined in recent decades and is on the brink of extinction in large parts of its distribution area (GEIST 2010, DENIC & GEIST 2017). The bottleneck appears to be the post-parasitic phase, during which juveniles depend on well sorted, stable substrates with con-

stantly high oxygen supply to the interstitial zone. At present, land-use changes and modifications of stream habitats have resulted in altered discharge regimes and increased siltation rates in fpm rivers. As a consequence, juvenile habitats are degraded due to a clogging of macropores in the interstitial zone by fine sediments with subsequent reduction of oxygen supply (HRUSKA 1999, GEIST & AUERSWALD 2007, ÖSTERLING et al. 2008, SCHEDER et al. 2015).

Habitat restoration is time consuming, keeping the complexity of various interacting factors in mind. This resulted in several fpm breeding projects all over Europe designed as short-term conservation measures to preserve the scattered and overaged populations. Two basic methodological approaches are established for fpm breeding (BUDDENSIEK 1995, GUM et al. 2011, LANGE & SELHEIM 2011, EYBE et al.

2013): (1) a lab-based method, where mussels are reared in artificial raceways and mainly fed with commercial algal diets, and (2) a semi-natural approach, which can include a short lab phase in the beginning of the breeding cycle until the juveniles reach the first millimetre. However, in the semi-natural approach, mussels are reared in field cage systems most of the time, which are exposed to the natural environment. In these field cages, fpm necessarily depend on natural food resources transported with the water current and are subject to natural variations in habitat conditions such as water flow, water chemistry and temperature (BUDDENSIEK 1995, GUM et al. 2011).

Both methods are well established and allow successful production of juvenile fpm. Which approach is preferred in a breeding programme mainly depends

on local circumstances such as funding resources, access to lab facilities or quality of rearing habitats. Consequently, even breeding programmes based on the same approach often differ in methodological details. Up to date, scientific evaluations of methods implemented in single breeding programmes are scarce, though this could further improve efficacy and efficiency in fpm breeding. In our experiment, we focused on the comparison of Buddensiek cages and sediment boxes, two different field cages currently used for the rearing of early post-parasitic life stages in semi-natural fpm propagation. Our purpose was to determine juvenile mussel performance in terms of growth and survival rates in the two field cages under different habitat conditions as well as to compare handling effort for both field cages.

MATERIAL AND METHODS

STUDY AREA

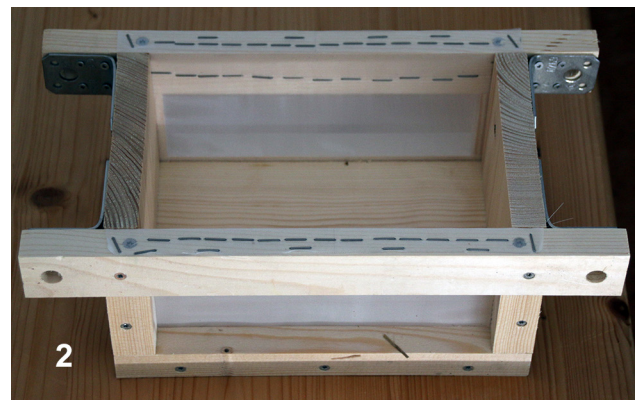
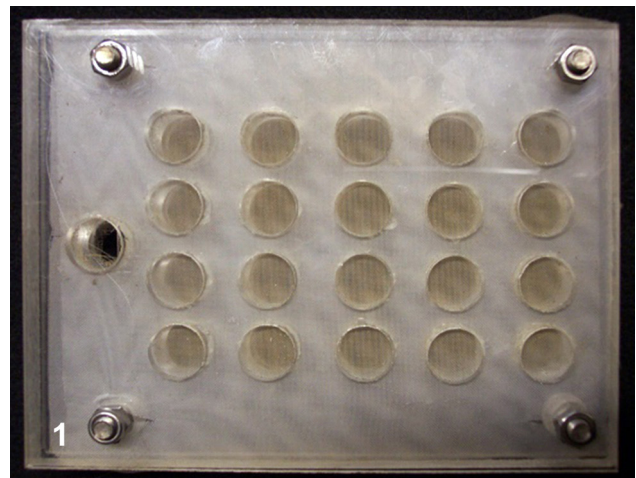
The study area is situated in the southern part of the Bavarian Forest in South-Eastern Germany close to the Austrian and Czech borderlines. With the Ilz (IL), Wolfsteiner Ohe (WO), Grosse Ohe (GO) and Kleine Ohe (KO) four study streams were selected, which all belong to the Danube catchment. All streams still host fpm populations, though they are small and with low natural recruitment. Their selection aimed at covering the typical range of fpm stream size in the study area, i.e. stream widths 2–30 m as well as a gradient in rearing habitat quality.

STUDY DESIGN

In total, 10 study sites were selected, three in each study stream except for river WO, which contained only one study site. At each study site, three Buddensiek cages (Fig. 1) and three sediment boxes (Fig. 2) were installed. The sediment boxes were closed with a plastic lid. Each field cage was stocked with 30 1+ fpm juveniles, which had been cultured in a breeding programme under identical conditions for one year. We only used juveniles from the fpm stock native to the respective study stream, where possible (rivers GO and KO). No 1+ juveniles were available from rivers WO and IL. Juveniles from river KO were used in rivers WO and IL, as it is known from former experiments (e.g. DENIC et al. 2015 and unpublished data) that this stock performs well there.

Before the experiment started in June 2016, juvenile shell lengths were determined. Mean juvenile shell length was 1.57 mm and ranged from 0.38–3.94 mm. Shell length and survival were investigated after three months and after 12 months, i.e. at the end of the summer growing season and in the follow-

ing spring. Juvenile shell length was determined by measuring the maximum total shell length ($\pm 2 \mu\text{m}$) using a binocular microscope connected to the LAS V4.8 software programme (Leica, Wetzlar, Germany). Growth rate was defined as the ratios of



Figs 1–2. Captive breeding elements used in this experiment: 1 – Buddensiek cage, 2 – sediment box



the pooled maximal shell lengths of all living individuals at specific sites at different time points.

All field cages were exposed to the free-flowing water and with mesh surfaces placed vertically to the current. Mesh size was 300 μm in all cages. Buddensiek cages were stocked with one juvenile per chamber and installed a few centimetres above the river substrate. Sediment boxes were placed on the river substrate. Sediment in the boxes consisted of detritus, which was added in a thin layer of a few millimetres. Over time more material, suspended in the river water, settled inside the sediment boxes. The surface of all field cages was brushed in biweekly intervals as long as water temperature was above 8 °C. Sediment boxes were opened and cleaned every eight weeks. If water temperature dropped below 8 °C, sediment boxes were brushed every six weeks but not opened at all. Additional cleaning took place directly after events with increased sediment transport, such as thunderstorms. If study sites were not accessible due to ice cover or elevated water levels, cleaning was carried out as soon as possible after the scheduled cleaning dates. Handling time was measured for each sediment box and Buddensiek cage during controls of mussel performance and cleaning. Time measurement for mussel performance controls included opening and closing of field cages, survival control and measurement of maximum total shell length. Documentation of cleaning time started with

RESULTS

A weak correlation was observed between initial shell length and survival of juveniles ($r = 0.3052034$; $p = 0.02218$), but mean initial shell length did not differ between Buddensiek cages and sediment boxes (Mann-Whitney-U-test, $p = 0.7991$). After three and 12 months, mean shell lengths differed significantly between Buddensiek cages (3.16 and 3.69 mm) and sediment boxes (3.3 and 3.9 mm) (Mann-Whitney-U-test, $p = 0.00487$ and $p = 0.0002904$).

Total survival rates were significantly higher in Buddensiek cages than in sediment boxes, even though survival over the summer growing season and over winter were comparable in both field cages. Survival in sediment boxes showed a stronger variation with lower minimum survival rates in both periods (Fig. 3). Overall, survival ranged between 40–93% in Buddensiek cages and 0–87% in sediment boxes. Survival rates were higher over winter than during the growing season in both field cages. Stream specific analysis of survival draws a similar picture, with generally high survival in Buddensiek cages and a broad, stream specific variation in sediment boxes. In every study stream, median survival rate was lower in sediment boxes compared

entering the stream, included brushing the surfaces of field cages and ended with leaving the stream channel. As a consequence, the measured time interval included handling of three field cages. Handling time for a single field cage was calculated by dividing the measured time by three. In case of sediment boxes, cleaning time could also include opening, cleaning the inside and closing the box again.

Electric Conductivity ($\mu\text{S}/\text{cm}$), Oxygen content (mg/L), Oxygen Saturation (%) and Turbidity (FNU) were measured at each cleaning date with a multi-parameter handheld meter (Hanna Instruments, Woonsocket, USA). Water temperature was measured hourly with HOBO Pendant Temperature/Light Data Loggers (Onset, Bourne, USA).

Data analyses were performed with RStudio 1.0.143 (RSTUDIO TEAM 2016). Statistical differences between physicochemical parameters, survival and growth rates were analysed with ANOVA and Tukey's post-hoc tests, if variances were homogenous using the auxiliary packages car and TukeyC (FOX & WEISBERG 2011, FARIA et al. 2017). If variances were not homogenous, Kruskal-Wallis test with subsequent Mann-Whitney-U-tests with Bonferroni correction were computed using the stats package (R CORE TEAM 2017). The latter was also used for calculation of Spearman's rank correlation between initial shell length of juveniles and total survival rates.

to Buddensiek cages except for study stream GO (Fig. 4).

In contrast to total survival, total growth rates did not differ between field cages. However, variation was generally strong ranging from 63–236% in

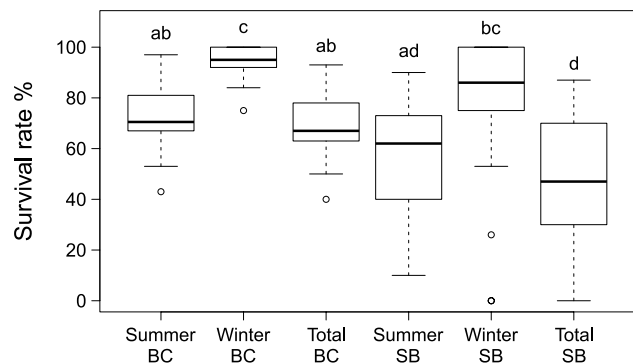


Fig. 3. Box and Whisker plots (Whisker – 0.05 and 0.95 percentiles, Box – lower quartile, median and upper quartile, circles – outliers) of survival rates in Buddensiek cages (BC) and sediment boxes (SB) between June–September 2016 (Summer), October 2016–May 2017 (Winter) and between June 2016–May 2017 (Total). Letters indicate significant differences at $p < 0.05$

sediment boxes and 53–262% in Buddensiek cages. Even though juveniles grew over the winter, the main growth was observed over the summer period. Growth rate was also strongly stream dependent with the fastest growth in study stream IL (Fig. 5). Though growth did not differ significantly between field cages, it was a little higher in Buddensiek cages except for river WO.

Turbidity, water temperature and specific conductivity revealed significant differences between study streams. In all cases KO had the highest mean values with 9.1 FNU, 10.0 °C and 157 $\mu\text{S}/\text{cm}$ as well as the highest maximum values. In contrast, WO had the lowest mean values for these three parameters with

4.2 FNU, 7.6 °C and 88 $\mu\text{S}/\text{cm}$. Table 1 provides an overview over the range in the measured parameters during the experiment.

At control dates, average handling time of sediment boxes was always lower than of Buddensiek cages. For both field cages, handling time was lowest at the start of the experiment with 21 min (sediment boxes) and 26 min (Buddensiek cages) and highest after the summer growing season with 37 min and 54 min, respectively. At cleaning dates, field cages did not differ in handling time as long as only the surface was brushed. When sediment boxes were opened and cleaned inside, average handling time increased from 93 s to 12 min 44 s.

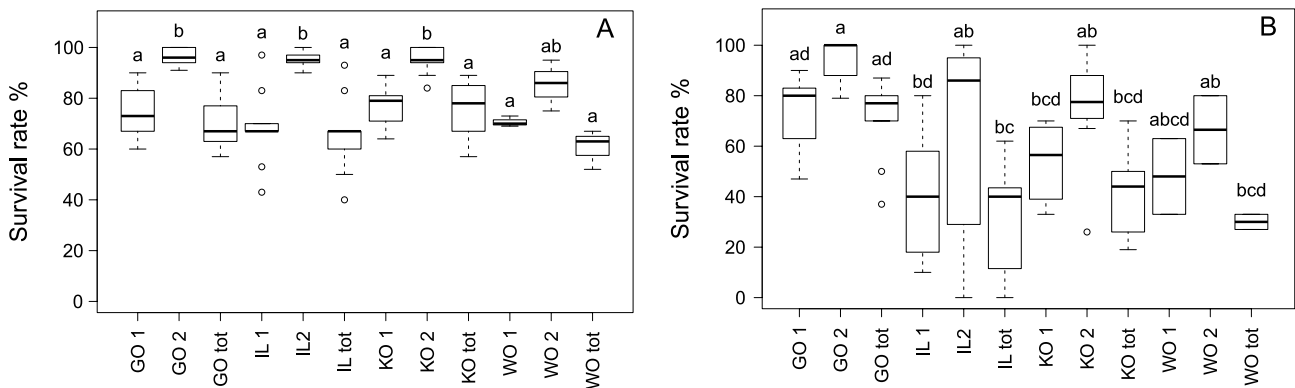


Fig. 4. Box and Whisker plots (Whisker – 0.05 and 0.95 percentiles, Box – lower quartile, median and upper quartile, circles – outliers) of stream specific survival in Buddensiek cages (A) and sediment boxes (B). GO – Große Ohe, IL – Ilz, KO – Kleine Ohe, WO – Wolfsteiner Ohe. 1 – survival June 2016–September 2016, 2 – October 2016–May 2017, tot – total survival June 2016–May 2017. Letters indicate significant differences at $p < 0.05$

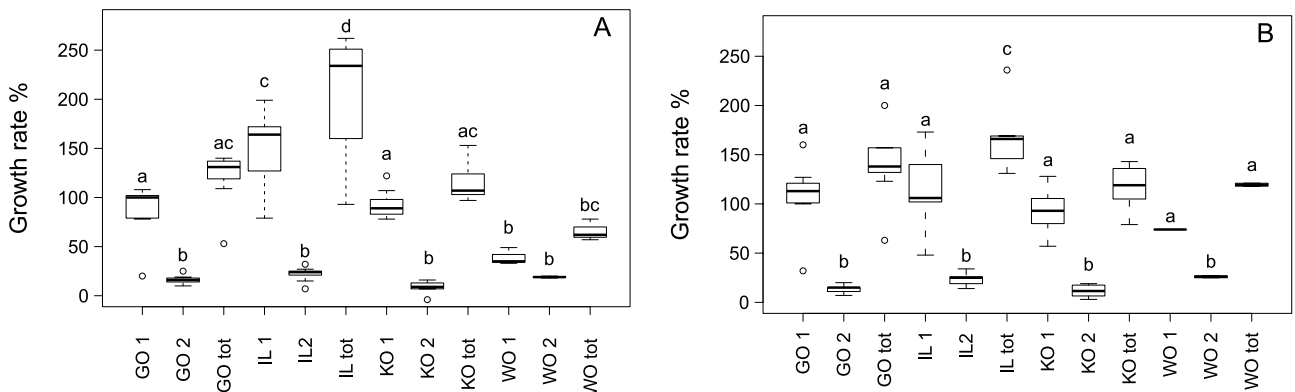


Fig. 5. Box and Whisker plots (Whisker – 0.05 and 0.95 percentiles, Box – lower quartile, median and upper quartile, circles – outliers) of stream specific growth rates in Buddensiek cages (A) and sediment boxes (B). GO – Große Ohe, IL – Ilz, KO – Kleine Ohe, WO – Wolfsteiner Ohe. 1 – growth rate June 2016–September 2016, 2 – growth rate October 2016–May 2017, tot – total growth rate June 2016–May 2017. Letters indicate significant differences at $p < 0.05$

Table 1. Arithmetic means and standard deviations of physicochemical parameters measured in study streams during exposure of juvenile freshwater pearl mussels between June 2016 and May 2017

Study stream	Water temperature [°C]	pH	Specific conductivity [$\mu\text{S}/\text{cm}$]	Dissolved oxygen saturation [%]	Dissolved oxygen content [mg/l]	Turbidity [FNU]
GO	9.3 \pm 6.1	6.9 \pm 0.4	122 \pm 19	92.5 \pm 4.4	9.3 \pm 0.7	5.6 \pm 2.1
IL	8.9 \pm 6.4	7.1 \pm 0.3	117 \pm 15	91.1 \pm 13.1	8.9 \pm 1.0	5.7 \pm 2.7
KO	10.0 \pm 6.7	7.0 \pm 0.4	157 \pm 9	90.5 \pm 7.9	9.1 \pm 0.8	9.1 \pm 4.5
WO	7.6 \pm 5.7	6.8 \pm 0.3	88 \pm 14	99.6 \pm 9.7	9.9 \pm 0.8	4.2 \pm 3.4



DISCUSSION

Two semi-natural rearing methods using Buddensiek cages and sediment boxes were compared. Both are suitable for rearing of juvenile freshwater pearl mussels. However, suitability, especially of sediment boxes, strongly depends on ambient habitat conditions at exposure sites. Where suitable, the field cages are work-saving rearing options for juvenile fpm.

Among others, [LANGE & SELHEIM \(2011\)](#) pointed out that winter survival of juvenile 0+ fpm is strongly correlated to a minimal shell length of 1 mm before the first winter. [SCHARTUM et al. \(2017\)](#) and [ARAUJO et al. \(2018\)](#) showed that severe morphological changes in the feeding organs, which are often unsuccessful, probably explain this observation. As a consequence, 1+ juveniles were chosen for the experiment, of which the majority already had reached a shell length of more than 1 mm when the experiment started, to exclude size related mortalities. The weak correlation between shell length and survival as well as the non-significant size differences between Buddensiek cages and sediment boxes in our experiment corroborate these findings and allow to attribute differences in survival rates mainly to field cages associated with habitat conditions.

Overall, juvenile performance was better in Buddensiek cages than in sediment boxes due to a narrower range and higher median values of survival rates. Nevertheless, stream specific trends of survival and growth rates were highly similar in both field cages, i.e. the same driving factors determined juvenile performance. Physicochemical measurements and the status of native fpm populations in the study streams prove that investigated water quality parameters mainly meet the demands of fpm. Nevertheless, the physicochemical data together with some anecdotal observations provide some evidence that crucial parameters are not in the optimal range for fpm habitats everywhere. In river KO, specific conductivity and turbidity were significantly higher than in the other study streams. Increased siltation may have reduced perfusion of field cages and consequently oxygen supply to juvenile fpm due to clogging of the gauze, reducing survival rates in this stream. Our results support findings of [SCHMIDT & VANDRÉ \(2010\)](#) that Buddensiek cages allow good juvenile performance in impacted habitats. Impacts may be stronger in sediment boxes, where juveniles are kept in one large compartment compared to many small ones in Buddensiek cages. As a consequence, oxygen depleted areas are more likely to occur in sediment boxes. Furthermore, strong algal growth was observed in several sediment boxes in river KO, probably favoured by elevated specific conductivity. Algae were also observed in two study sites at river IL down-

stream of reservoirs, where survival was very low. However, growth rates were nearly twice as high as at the other study sites, maybe due to increased food production in reservoirs. Survival was expected to be higher in river WO and the third study site in river IL, due to former results in the fpm breeding programme and the study of [DENIC et al. \(2015\)](#) as well as results of physicochemical measurements, which was not the case. This implies that peak events, which were not detected by routine measurements of physicochemical parameters, may have reduced juvenile performance ([HASTIE et al. 2001](#), [DENIC & GEIST 2015](#)). Indeed, strong thunderstorms were extremely frequent over the summer of 2016. As the majority of mortalities occurred between two cleaning dates in this period, the introduction of some pollutant during one such event may explain these unexpected results. Finally, none of the observations discussed above was made in river GO, which was the river with the highest survival in the experiment and the only study stream, where performance was better in sediment boxes than in Buddensiek cages. Consequently, the closer physicochemical habitat conditions at an exposure site fit to the demands of fpm and the more constant conditions stay in that range, the better juveniles perform in sediment boxes.

Local habitat conditions are a key factor for the choice of the cage type for semi-natural rearing of fpm. Nevertheless, parameters such as handling effort should not be neglected before the choice of a specific field cage. As the results in this study show, handling effort for cleaning is higher per sediment box than per Buddensiek cage. However, control of juvenile performance is more time consuming in Buddensiek cages and strongly increases with increasing numbers of juvenile fpm. Therefore, the higher the number of juveniles to be reared the more efficient it is to work with sediment boxes, as more juveniles can be raised per box and handling of boxes is not correlated with the number of mussels inside. Moreover, the purpose of the project influences the suitability of the field cage. Due to the possibility of separating juveniles in single chambers of Buddensiek cages, they are more preferable for bioindication studies, as individual performance can be investigated, whereas in sediment boxes only average values can be calculated.

To conclude, both field cages are suitable for rearing of juvenile fpm. However, their suitability is strongly related to local habitats, number of juveniles and purpose of the project. Therefore, there is no general recommendation for one of the field cages but an individual decision based on the parameters discussed above is necessary for each project or even study site. If some of the underlying parameters are unknown, a pre-study is recommended.



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